TITLE OF THE INVENTION

HIGHLY CONSERVED GENES AND THEIR USE TO GENERATE SPECIES-SPECIFIC, GENUS-SPECIFIC, FAMILY-SPECIFIC, GROUP-SPECIFIC AND UNIVERSAL NUCLEIC ACID PROBES AND AMPLIFICATION PRIMERS TO RAPIDLY DETECT AND IDENTIFY ALGAL, ARCHAEAL, BACTERIAL, FUNGAL AND PARASITICAL MICROORGANISMS FROM CLINICAL SPECIMENS FOR DIAGNOSIS

BACKGROUND OF THE INVENTION

Classical methods for the identification of microorganisms

Microorganisms are classically identified by their ability to utilize different substrates as a source of carbon and nitrogen through the use of biochemical tests such as the API20E™ system (bioMérieux). For susceptibility testing, clinical microbiology laboratories use methods including disk diffusion, agar dilution and broth microdilution. Although identifications based on biochemical tests and antibacterial susceptibility tests are cost-effective, generally two days are required to obtain preliminary results due to the necessity of two successive overnight incubations to identify the bacteria from clinical specimens as well as to determine their susceptibility to antimicrobial agents. There are some commercially available automated systems (i.e. the MicroScan™ system from Dade Behring and the Vitek™ system from bioMérieux) which use sophisticated and expensive apparatus for faster microbial identification and susceptibility testing (Stager and Davis, 1992, Clin. Microbiol. Rev. 5:302-327). These systems require shorter incubation periods, thereby allowing most bacterial identifications and susceptibility testing to be performed in less than 6 hours. Nevertheless, these

faster systems always require the primary isolation of the bacteria or fungi as a pure culture, a process which takes at least 18 hours for a pure culture or 2 days for a mixed culture. So, the shortest time from sample reception to identification of the pathogen is around 24 hours. Moreover, fungi other than yeasts are often difficult or very slow to grow from clinical specimens. Identification must rely on labor-intensive techniques such as direct microscopic examination of the specimens and by direct and/or indirect immunological assays. Cultivation of most parasites is impractical in the clinical laboratory. Hence, microscopic examination of the specimen, a few immunological tests and clinical symptoms are often the only methods used for an identification that frequently remains presumptive.

The fastest bacterial identification system, the autoSCAN-Walk-Away™ system (Dade Behring) identifies both gram-negative and gram-positive bacterial species from standardized inoculum in as little as 2 hours and gives susceptibility patterns to most antibiotics in 5 to 6 hours. However, this system has a particularly high percentage (i.e. 3.3 to 40.5%) of non-conclusive identifications with bacterial species other than *Enterobacteriaceae* (Croizé J., 1995, Lett. Infectiol. **10**:109-113; York *et al.*, 1992, J. Clin. Microbiol. **30**:2903-2910). For *Enterobacteriaceae*, the percentage of non-conclusive identifications was 2.7 to 11.4%. The list of microorganisms identified by commercial systems based on classical identification methods is given in Table 15.

A wide variety of bacteria and fungi are routinely isolated and identified from clinical specimens in microbiology laboratories. Tables 1 and 2 give the incidence for the most commonly isolated bacterial and fungal pathogens from various types of clinical specimens. These pathogens are the main organisms associated with nosocomial and community-acquired human infections and are therefore considered the most clinically important.

Clinical specimens tested in clinical microbiology laboratories

Most clinical specimens received in clinical microbiology laboratories are urine and blood samples. At the microbiology laboratory of the Centre Hospitalier de l'Université Laval (CHUL), urine and blood account for approximately 55% and 30% of the specimens received, respectively (Table 3). The remaining 15% of clinical specimens comprise various biological fluids including sputum, pus, cerebrospinal fluid, synovial fluid, and others (Table 3). Infections of the urinary tract, the respiratory tract and the bloodstream are usually of bacterial etiology and require antimicrobial therapy. In fact, all clinical samples received in the clinical microbiology laboratory are tested routinely for the identification of bacteria and antibiotic susceptibility.

Conventional pathogen identification from clinical specimens

Urine specimens

The search for pathogens in urine specimens is so preponderant in the routine microbiology laboratory that a myriad of tests have been developed. However, the gold standard remains the classical semi-quantitative plate culture method in which 1 µL of urine is streaked on agar plates and incubated for 18-24 hours. Colonies are then counted to determine the total number of colony forming units (CFU) per liter of urine. A bacterial urinary tract infection (UTI) is normally associated with a bacterial count of 10^7 CFU/L or more in urine. However, infections with less than 10^7 CFU/L in urine are possible, particularly in patients with a high incidence of diseases or those catheterized (Stark and Maki, 1984, N. Engl. J. Med. **311**:560-564). Importantly, approximately 80% of urine specimens tested in clinical microbiology laboratories are considered negative (i.e. bacterial count of less than 10^7 CFU/L; Table 3). Urine specimens found positive by culture are further characterized using standard biochemical tests to identify the bacterial pathogen and are also tested for susceptibility to antibiotics. The biochemical and susceptibility testing normally require 18-24 hours of incubation.

Accurate and rapid urine screening methods for bacterial pathogens would allow a faster identification of negative specimens and a more efficient treatment and care management of patients. Several rapid identification methods (Uriscreen™, UTIscreen™, Flash Track™ DNA probes and others) have been compared to slower standard biochemical methods, which are based on culture of the bacterial pathogens. Although much faster, these rapid tests showed low sensitivities and poor specificities as well as a high number of false negative and false positive results (Koening *et al.*, 1992, J. Clin. Microbiol. **30**:342-345; Pezzlo *et al.*, 1992, J. Clin. Microbiol. **30**:640-684).

Blood specimens

The blood specimens received in the microbiology laboratory are always submitted for culture. Blood culture systems may be manual, semi-automated or completely automated. The BACTEC™ system (from Becton Dickinson) and the BacTAlert™ system (from Organon Teknika Corporation) are the two most widely used automated blood culture systems. These systems incubate blood culture bottles under optimal conditions for growth of most bacteria. Bacterial growth is monitored continuously to detect early positives by using highly sensitive bacterial growth detectors. Once growth is detected, a Gram stain is performed directly from the blood culture and then used to inoculate nutrient agar plates. Subsequently, bacterial identification and susceptibility testing are carried out from isolated bacterial colonies with automated systems as described previously. Blood culture bottles are normally reported as negative if no growth is detected after an incubation of 6 to 7 days. Normally, the vast majority of blood cultures are reported negative. For example, the percentage of negative blood cultures at the microbiology laboratory of the CHUL for the period February 1994-January 1995 was 93.1% (Table 3).

Other clinical samples

Upon receipt by the clinical microbiology laboratory, all body fluids other than blood and urine that are from normally sterile sites (i.e. cerebrospinal, synovial, pleural, pericardial and others) are processed for direct microscopic examination and subsequent culture. Again, most clinical samples are negative for culture (Table 3). In all these normally sterile sites, tests for the universal detection of algae, archaea, bacteria, fungi and parasites would be very useful .

Regarding clinical specimens which are not from sterile sites such as sputum or stool specimens, the laboratory diagnosis by culture is more problematic because of the contamination by the normal flora. The bacterial or fungal pathogens potentially associated with the infection are grown and separated from the colonizing microbes using selective methods and then identified as described previously. Of course, the DNA-based universal detection of bacteria would not be useful for the diagnosis of bacterial infections at these non-sterile sites. On the other hand, DNA-based assays for species or genus or family or group detection and identification as well as for the detection of antimicrobial agents resistance genes from these specimens would be very useful and would offer several advantages over classical identification and susceptibility testing methods.

DNA-based assays with any specimen

There is an obvious need for rapid and accurate diagnostic tests for the detection and identification of algae, archaea, bacteria, fungi and parasites directly from clinical specimens. DNA-based technologies are rapid and accurate and offer a great potential to improve the diagnosis of infectious diseases (Persing *et al.*, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.; Bergeron and Ouellette, 1995, Infection **23**:69-72; Bergeron and Ouellette, 1998, J Clin Microbiol. **36**:2169-72). The DNA probes and amplification primers which are objects of the present invention are applicable for the detection and identification of algae, archaea, bacteria, fungi, and parasites directly from any clinical specimen such as blood,

urine, sputum, cerebrospinal fluid, pus, genital and gastro-intestinal tracts, skin or any other type of specimens (Table 3). These assays are also applicable to detection from microbial cultures (e.g. blood cultures, bacterial or fungal colonies on nutrient agar, or liquid cell cutures in nutrient broth). The DNA-based tests proposed in this invention are superior in terms of both rapidity and accuracy to standard biochemical methods currently used for routine diagnosis from any clinical specimens in microbiology laboratories. Since these tests can be performed in one hour or less, they provide the clinician with new diagnostic tools which should contribute to a better management of patients with infectious diseases. Specimens from sources other than humans (e.g. other primates, birds, plants, mammals, farm animals, livestock, food products, environment such as water or soil, and others) may also be tested with these assays.

A high percentage of culture-negative specimens

Among all the clinical specimens received for routine diagnosis, approximately 80% of urine specimens and even more (around 95%) for other types of normally sterile clinical specimens are negative for the presence of bacterial pathogens (Table 3). It would also be desirable, in addition to identify bacteria at the species or genus or family or group level in a given specimen, to screen out the high proportion of negative clinical specimens with a DNA-based test detecting the presence of any bacterium (i.e. universal bacterial detection). As disclosed in the present invention, such a screening test may be based on DNA amplification by PCR of a highly conserved genetic target found in all bacteria. Specimens negative for bacteria would not be amplified by this assay. On the other hand, those that are positive for any bacterium would give a positive amplification signal. Similarly, highly conserved genes of fungi and parasites could serve not only to identify particular species or genus or family or group but also to detect the presence of any fungi or parasite in the specimen.

Towards the development of rapid DNA-based diagnostic tests

A rapid diagnostic test should have a significant impact on the management of infections. DNA probe and DNA amplification technologies offer several advantages over conventional methods for the identification of pathogens and antimicrobial agents resistance genes from clinical samples (Persing *et al.*, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.; Ehrlich and Greenberg, 1994, PCR-based Diagnostics in Infectious Disease, Blackwell Scientific Publications, Boston, MA). There is no need for culture of the pathogens, hence the organisms can be detected directly from clinical samples, thereby reducing the time associated with the isolation and identification of pathogens. Furthermore, DNA-based assays are more accurate for microbial identification than currently used phenotypic identification systems which are based on biochemical tests and/or microscopic examination. Commercially available DNA-based technologies are currently used in clinical microbiology laboratories, mainly for the detection and identification of fastidious bacterial pathogens such as *Mycobacterium tuberculosis*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae* as well as for the detection of a variety of viruses (Tang Y. and Persing D. H., Molecular detection and identification of microorganisms, *In*: P. Murray *et al.*, 1999, Manual of Clinical Microbiology, ASM press, 7th edition, Washington D.C.). There are also other commercially available DNA-based assays which are used for culture confirmation assays.

Others have developed DNA-based tests for the detection and identification of bacterial pathogens which are objects of the present invention, for example: *Staphylococcus* sp. (US patent serial no. 5,437,978), *Neisseria* sp. (US patent serial no. 5,162,199 and European patent serial no. 0,337,896,131) and *Listeria monocytogenes* (US patent serial nos. 5,389,513 and 5,089,386). However, the diagnostic tests described in these patents are based either on rRNA genes or on genetic targets different from those described in the present invention. To our knowledge there are only four patents published by others mentioning the use of

any of the four highly conserved gene targets described in the present invention for diagnostic purposes (PCT international publication number WO92/03455 and WO00/14274, European patent publication number 0 133 671 B1, and European patent publication number 0 133 288 A2). WO92/03455 is focused on the inhibition of *Candida* species for therapeutic purposes. It describes antisense oligonucleotide probes hybridizing to *Candida* messenger RNA. Two of the numerous mRNA proposed as targets are coding for translation elongation factor 1 (tef1) and the beta subunit of ATPase. DNA amplification or hybrization are not under the scope of their invention and although diagnostic use is briefly mentioned in the body of the application, no specific claim is made regarding diagnostics. WO00/14274 describes the use of bacterial *recA* gene for identification and speciation of bacteria of the *Burkholderia cepacia* complex. Specific claims are made on a method for obtaining nucleotide sequence information for the *recA* gene from the target bacteria and a following comparison with a standard library of nucleotide sequence information (claim 1), and on the use of PCR for amplification of the *recA* gene in a sample of interest (claims 4 to 7, and 13). However, the use of a discriminatory restriction enzyme in a RFLP procedure is essential to fulfill the speciation and WO00/14274 did not mention that multiple *recA* probes could be used simultaneously. Patent EP 0 133 288 A2 describes and claims the use of bacterial *tuf* (and *fus*) sequence for diagnostics based on hybridization of a *tuf* (or *fus*) probe with bacterial DNA. DNA amplification is not under the scope of EP 0 133 288 A2. Nowhere it is mentioned that multiple *tuf* (or *fus*) probes could be used simultaneously. No mention is made regarding speciation using *tuf* (or *fus*) DNA nucleic acids and/or sequences. The sensitivities of the *tuf* hybrizations reported are $1x10^6$ bacteria or 1-100 ng of DNA. This is much less sensitive than what is achieved by our assays using nucleic acid amplification technologies.

Although there are phenotypic identification methods which have been used for more than 125 years in clinical microbiology laboratories, these methods do not provide information fast enough to be useful in the initial management of patients.

There is a need to increase the speed of the diagnosis of commonly encountered bacterial, fungal and parasitical infections. Besides being much faster, DNA-based diagnostic tests are more accurate than standard biochemical tests presently used for diagnosis because the microbial genotype (e.g. DNA level) is more stable than the phenotype (e.g. physiologic level).

Bacteria, fungi and parasites encompass numerous well-known microbial pathogens. Other microorganisms could also be pathogens or associated with human diseases. For example, achlorophylious algae of the *Prototheca* genus can infect humans. Archae, especially methanogens, are present in the gut flora of humans (Reeve, J.H., 1999, J. Bacteriol. **181**:3613-3617). However, methanogens have been associated to pathologic manifestations in the colon, vagina, and mouth (Belay *et al.*, 1988, Appl. Enviro. Microbiol. **54**:600-603; Belay *et al.*, 1990, J. Clin. Microbiol. **28**:1666-1668; Weaver *et al.*, 1986, Gut **27**:698-704).

In addition to the identification of the infectious agent, it is often desirable to identify harmful toxins and/or to monitor the sensitivity of the microorganism to antimicrobial agents. As revealed in this invention, genetic identification of the microorganism could be performed simultaneously with toxin and antimicrobial agents resistance genes.

Knowledge of the genomic sequences of algal, archaeal, bacterial, fungal and parasitical species continuously increases as testified by the number of sequences available from public databases such as GenBank. From the sequences readily available from those public databases, there is no indication therefrom as to their potential for diagnostic purposes. For determining good candidates for diagnostic purposes, one could select sequences for DNA-based assays for (i) the species-specific detection and identification of commonly encountered bacterial, fungal and parasitical pathogens, (ii) the genus-specific detection and identification of commonly encountered bacterial, fungal or parasitical pathogens, (iii) the family-specific detection and identification of commonly encountered bacterial, fungal or parasitical pathogens, (iv) the group-specific detection and identification of commonly encountered bacterial, fungal or parasitical pathogens, (v) the

universal detection of algal, archaeal, bacterial, fungal or parasitical pathogens, and/or (vi) the specific detection and identification of antimicrobial agents resistance genes, and/or (vii) the specific detection and identification of bacterial toxin genes. All of the above types of DNA-based assays may be performed directly from any type of clinical specimens or from a microbial culture.

In our assigned U.S. patent 6,001,564 and our WO98/20157 patent publication, we described DNA sequences suitable for (i) the species-specific detection and identification of clinically important bacterial pathogens, (ii) the universal detection of bacteria, and (iii) the detection of antimicrobial agents resistance genes.

The WO98/20157 patent publication describes proprietary *tuf* DNA sequences as well as *tuf* sequences selected from public databases (in both cases, fragments of at least 100 base pairs), as well as oligonucleotide probes and amplification primers derived from these sequences. All the nucleic acid sequences described in that patent publication can enter in the composition of diagnostic kits or products and methods capable of a) detecting the presence of bacteria and fungi b) detecting specifically at the species, genus, family or group levels, the presence of bacteria and fungi and antimicrobial agents resistance genes associated with these pathogens. However, these methods and kits need to be improved, since the ideal kit and method should be capable of diagnosing close to 100% of microbial pathogens and associated antimicrobial agents resistance genes and toxins genes. For example, infections caused by *Enterococcus faecium* have become a clinical problem because of its resistance to many antibiotics. Both the detection of these bacteria and the evaluation of their resistance profiles are desirable. Besides that, novel DNA sequences (probes and primers) capable of recognizing the same and other microbial pathogens or the same and additional antimicrobial agents resistance genes are also desirable to aim at detecting more target genes and complement our earlier patent applications.

The present invention improves the assigned application by disclosing new proprietary *tuf* nucleic acids and/or sequences as well as describing new ways to

obtain *tuf* nucleic acids and/or sequences. In addition we disclose new proprietary *atpD* and *recA* nucleic acids and/or sequences. In addition, new uses of *tuf*, *atpD* and *recA* DNA nucleic acids and/or sequences selected from public databases (Table 11) are disclosed.

Highly conserved genes for identification and diagnostics

Highly conserved genes are useful for identification of microorganisms. For bacteria, the most studied genes for identification of microorganisms are the universally conserved ribosomal RNA genes (rRNA). Among those, the principal targets used for identification purposes are the small subunit (SSU) ribosomal 16S rRNA genes (in prokaryotes) and 18S rRNA genes (in eukaryotes) (Relman and Persing, Genotyping Methods for Microbial Identification, *In*: D.H. Persing, 1996, PCR Protocols for Emerging Infectious Diseases, ASM Press, Washington D.C.). The rRNA genes are also the most commonly used targets for universal detection of bacteria (Chen *et al.*, 1988, FEMS Microbiol. Lett. **57**:19-24; McCabe *et al.*, 1999, Mol. Genet. Metabol. **66**:205-211) and fungi (Van Burik *et al.*, 1998, J. Clin. Microbiol. **36**:1169-1175).

However, it may be difficult to discriminate between closely related species when using primers derived from the 16S rRNA. In some instances, 16S rRNA sequence identity may not be sufficient to guarantee species identity (Fox *et al.*, 1992, Int. J. Syst. Bacteriol. **42**:166-170) and it has been shown that inter-operon sequence variation as well as strain to strain variation could undermine the application of 16S rRNA for identification purposes (Clayton *et al.*, 1995, Int. J. Syst. Bacteriol. **45**:595-599). The heat shock proteins (HSP) are another family of very conserved proteins. These ubiquitous proteins in bacteria and eukaryotes are expressed in answer to external stress agents. One of the most described of these HSP is HSP 60. This protein is very conserved at the amino acid level, hence it has been useful for phylogenetic studies. Similar to 16S rRNA, it would be difficult to

discriminate between species using the HSP 60 nucleotide sequences as a diagnostic tool. However, Goh *et al.* identified a highly conserved region flanking a variable region in HSP 60, which led to the design of universal primers amplifying this variable region (Goh *et al.*, US patent serial no. 5,708,160). The sequence variations in the resulting amplicons were found useful for the design of species-specific assays.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a specific, ubiquitous and sensitive method using probes and/or amplification primers for determining the presence and/or amount of nucleic acids:

- from any algal, archaeal, bacterial, fungal or parasitical species

in any sample suspected of containing said nucleic acids, and optionally,

- from specific microbial species or genera selected from the group consisting of the species or genera listed in Table 4, and optionally,
- from an antimicrobial agents resistance gene selected from the group consisting of the genes listed in Table 5, and optionally,
- from a toxin gene selected from the group consisting of the genes listed in Table 6,

wherein each of said nucleic acids or a variant or part thereof comprises a selected target region hybridizable with said probes or primers;

said method comprising the steps of contacting said sample with said probes or primers and detecting the presence and/or amount of hybridized probes or amplified products as an indication of the presence and/or amount of said any

microbial species, specific microbial species or genus or family or group and antimicrobial agents resistance gene and/or toxin gene.

In a specific embodiment, a similar method directed to each specific microbial species or genus or family or group detection and identification, antimicrobial agents resistance genes detection, toxin genes detection, and universal bacterial detection, separately, is provided.

In a more specific embodiment, the method makes use of DNA fragments from conserved genes (proprietary sequences and sequences obtained from public databases), selected for their capacity to sensitively, specifically and ubiquitously detect the targeted algal, archaeal, bacterial, fungal or parasitical nucleic acids.

In a particularly preferred embodiment, oligonucleotides of at least 12 nucleotides in length have been derived from the longer DNA fragments, and are used in the present method as probes or amplification primers. To be a good diagnostic candidate, an oligonucleotide of at least 12 nucleotides should be capable of hybridizing with nucleic acids from given microorganism(s), and with substantially all strains and representatives of said microorganism(s); said oligonucleotide being species-, or genus-, or family-, or group-specific or universal.

In another particularly preferred embodiment, oligonucleotides primers and probes of at least 12 nucleotides in length are designed for their specificity and ubiquity based upon analysis of our databases of *tuf*, *atpD* and *recA* sequences. These databases are generated using both proprietary and public sequence information. Altogether, these databases form a sequence repertory useful for the design of primers and probes for the detection and identification ofalgal, archaeal, bacterial, fungal and parasitical microorganisms. The repertory can also be subdivided into subrepertories for sequence analysis leading to the design of various primers and probes.

The *tuf*, *atpD* and *recA* sequences databases as a product to assist the design of oligonucleotides primers and probes for the detection and identification ofalgal, archaeal, bacterial, fungal and parasitical microorganisms are also covered.

The proprietary oligonucleotides (probes and primers) are also another object of this invention.

Diagnostic kits comprising probes or amplification primers such as those for the detection of a microbial species or genus or family or phylum or group selected from the following list consisting of *Abiotrophia adiacens*, *Acinetobacter baumanii*, *Actinomycetae*, *Bacteroides*, *Cytophaga* and *Flexibacter* phylum, *Bacteroides fragilis*, *Bordetella pertussis*, *Bordetella* sp., *Campylobacter jejuni* and *C. coli*, *Candida albicans*, *Candida dubliniensis*, *Candida glabrata*, *Candida guilliermondii*, *Candida krusei*, *Candida lusitaniae*, *Candida parapsilosis*, *Candida tropicalis*, *Candida zeylanoides*, *Candida* sp., *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium* sp., *Corynebacterium* sp., *Crypococcus neoformans*, *Cryptococcus* sp., *Cryptosporidium parvum*, *Entamoeba* sp., *Enterobacteriaceae* group, *Enterococcus casseliflavus-flavescens-gallinarum* group, *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus gallinarum*, *Enterococcus* sp., *Escherichia coli* and *Shigella* sp. group, *Gemella* sp., *Giardia* sp., *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Legionella* sp., *Leishmania* sp., *Mycobacteriaceae* family, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, platelets contaminants group (see Table 14), *Pseudomonas aeruginosa*, Pseudomonads group, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus saprophyticus*, *Staphylococcus* sp., *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus* sp., *Trypanosoma brucei*, *Trypanosoma cruzi*, *Trypanosoma* sp., *Trypanosomatidae* family, are also objects of the present invention.

Diagnostic kits further comprising probes or amplification primers for the detection of an antimicrobial agents resistance gene selected from the group listed in Table 5 are also objects of this invention.

Diagnostic kits further comprising probes or amplification primers for the detection of a toxin gene selected from the group listed in Table 6 are also objects of this invention.

Diagnostic kits further comprising probes or amplification primers for the detection of any other algal, archaeal, bacterial, fungal or parasitical species than those specifically listed herein, comprising or not comprising those for the detection of the specific microbial species or genus or family or group listed above, and further comprising or not comprising probes and primers for the antimicrobial agents resistance genes listed in Table 5, and further comprising or not comprising probes and primers for the toxin genes listed in Table 6 are also objects of this invention.

In a preferred embodiment, such a kit allows for the separate or the simultaneous detection and identification of the above-listed microbial species or genus or family or group; or universal detection of algae, archaea, bacteria, fungi or parasites; or antimicrobial agents resistance genes; or toxin genes; or for the detection of any microorganism (algae, archaea, bacteria, fungi or parasites).

In the above methods and kits, probes and primers are not limited to nucleic acids and may include, but are not restricted to analogs of nucleotides such as: inosine, 3-nitropyrrole nucleosides (Nichols *et al.*, 1994, Nature **369**:492-493), Linked Nucleic Acids (LNA) (Koskin *et al.*, 1998, Tetrahedron **54**:3607-3630), and Peptide Nucleic Acids (PNA) (Egholm *et al.*, 1993, Nature **365**:566-568).

In the above methods and kits, amplification reactions may include but are not restricted to: a) polymerase chain reaction (PCR), b) ligase chain reaction (LCR), c) nucleic acid sequence-based amplification (NASBA), d) self-sustained sequence replication (3SR), e) strand displacement amplification (SDA), f) branched DNA signal amplification (bDNA), g) transcription-mediated amplification (TMA), h) cycling probe technology (CPT), i) nested PCR, j) multiplex PCR, k) solid phase amplification (SPA), l) nuclease dependent signal amplification (NDSA), m) rolling circle amplification technology (RCA), n) Anchored strand displacement amplification, o) Solid-phase (immobilized) rolling circle amplification.

In the above methods and kits, detection of the nucleic acids of target genes may include real-time or post-amplification technologies. These detection

technologies can include, but are not limited to, fluorescence resonance energy transfer (FRET)-based methods such as adjacent hybridization to FRET probes (including probe-probe and probe-primer methods), TaqMan, Molecular Beacons, scorpions, nanoparticle probes and Sunrise (Amplifluor). Other detection methods include target genes nucleic acids detection via immunological methods, solid phase hybridization methods on filters, chips or any other solid support, whether the hybridization is monitored by fluorescence, chemiluminescence, potentiometry, mass spectrometry, plasmon resonance, polarimetry, colorimetry, or scanometry. Sequencing, including sequencing by dideoxy termination or sequencing by hybridization, e.g. sequencing using a DNA chip, is another possible method to detect and identify the nucleic acids of target genes.

In a preferred embodiment, a PCR protocol is used for nucleic acid amplification, in diagnostic method as well as in method of construction of a repertory of nucleic acids and deduced sequences.

In a particularly preferred embodiment, a PCR protocol is provided, comprising, an initial denaturation step of 1-3 minutes at 95 °C, followed by an amplification cycle including a denaturation step of one second at 95 °C and an annealing step of 30 seconds at 45-65°C, without any time allowed specifically for the elongation step. This PCR protocol has been standardized to be suitable for PCR reactions with most selected primer pairs, which greatly facilitates the testing because each clinical sample can be tested with universal, species-specific, genus-specific, antimicrobial agents resistance gene and toxin gene PCR primers under uniform cycling conditions. Furthermore, various combinations of primer pairs may be used in multiplex PCR assays.

It is also an object of the present invention that *tuf*, *atpD* and *recA* sequences could serve as drug targets and these sequences and means to obtain them revealed in the present invention can assist the screening, design and modeling of these drugs.

It is also an object of the present invention that *tuf*, *atpD* and *recA* sequences could serve for vaccine purposes and these sequences and means to obtain them

revealed in the present invention can assist the screening, design and modeling of these vaccines.

We aim at developing a universal DNA-based test or kit to screen out rapidly samples which are free of algal, archaeal, bacterial, fungal or parasitical cells. This test could be used alone or combined with more specific identification tests to detect and identify the above algal and/or archaeal and/or bacterial and/or fungal and/or parasitical species and/or genera and/or family and/or group and to determine rapidly the bacterial resistance to antibiotics and/or presence of bacterial toxins. Although the sequences from the selected antimicrobial agents resistance genes are available from public databases and have been used to develop DNA-based tests for their detection, our approach is unique because it represents a major improvement over current diagnostic methods based on bacterial cultures. Using an amplification method for the simultaneous or independent or sequential microbial detection-identification and antimicrobial resistance genes detection, there is no need for culturing the clinical sample prior to testing. Moreover, a modified PCR protocol has been developed to detect all target DNA sequences in approximately one hour under uniform amplification conditions. This procedure should save lives by optimizing treatment, should diminish antimicrobial agents resistance because less antibiotics will be prescribed, should reduce the use of broad spectrum antibiotics which are expensive, decrease overall health care costs by preventing or shortening hospitalizations, and side effects of drugs, and decrease the time and costs associated with clinical laboratory testing.

In another embodiment, sequence repertories and ways to obtain them for other gene targets are also an object of this invention, such is the case for the *hexA* nucleic acids and/or sequences of Streptococci.

In yet another embodiment, for the detection of mutations associated with antibiotic resistance genes, we built repertories to distinguish between point mutations reflecting only gene diversity and point mutations involved in resistance. Such repertories and ways to obtain them for *pbp1a*, *pbp2b* and *pbp2x* genes of sensitive and penicillin-resistant *Streptoccoccus pneumoniae* and also for *gyrA* and

parC gene fragments from various bacterial species are also an object of the present invention.

The diagnostic kits, primers and probes mentioned above can be used to identify algae, archaea, bacteria, fungi, parasites, antimicrobial agents resistance genes and toxin genes on any type of sample, whether said diagnostic kits, primers and probes are used for *in vitro* or *in situ* applications. The said samples may include but are not limited to: any clinical sample, any environment sample, any microbial culture, any microbial colony, any tissue, and any cell line.

It is also an object of the present invention that said diagnostic kits, primers and probes can be used alone or in conjunction with any other assay suitable to identify microorganisms, including but not limited to: any immunoassay, any enzymatic assay, any biochemical assay, any lysotypic assay, any serological assay, any differential culture medium, any enrichment culture medium, any selective culture medium, any specific assay medium, any identification culture medium, any enumeration cuture medium, any cellular stain, any culture on specific cell lines, and any infectivity assay on animals.

In the methods and kits described herein below, the oligonucleotide probes and amplification primers have been derived from larger sequences (i.e. DNA fragments of at least 100 base pairs). All DNA fragments have been obtained either from proprietary fragments or from public databases. DNA fragments selected from public databases are newly used in a method of detection according to the present invention, since they have been selected for their diagnostic potential.

In another embodiment, the amino acid sequences translated from the repertory of *tuf*, *atpD* and *recA* nucleic acids and/or sequences are also an object of the present invention.

It is clear to the individual skilled in the art that other oligonucleotide sequences appropriate for (i) the universal detection of algae, archaea, bacteria, fungi or parasites, (ii) the detection and identification of the above microbial species or genus or family or group, and (iii) the detection of antimicrobial agents resistance genes, and (iv) the detection of toxin genes, other than those listed in

Annexes I to III, XXI to XXII, XXXII to XXXVII, XXXIX to XLI, and XLIII to LIV may also be derived from the proprietary fragments or selected public database sequences. For example, the oligonucleotide primers or probes may be shorter or longer than the ones chosen; they may also be selected anywhere else in the proprietary DNA fragments or in the sequences selected from public databases; they may be also variants of the same oligonucleotide. If the target DNA or a variant thereof hybridizes to a given oligonucleotide, or if the target DNA or a variant thereof can be amplified by a given oligonucleotide PCR primer pair, the converse is also true; a given target DNA may hybridize to a variant oligonucleotide probe or be amplified by a variant oligonucleotide PCR primer. Alternatively, the oligonucleotides may be designed from any DNA fragment sequences for use in amplification methods other than PCR. Consequently, the core of this invention is the identification of universal, species-specific, genus-specific, family-specific, group-specific, resistance gene-specific, toxin gene-specific genomic or non-genomic DNA fragments which are used as a source of specific and ubiquitous oligonucleotide probes and/or amplification primers. Although the selection and evaluation of oligonucleotides suitable for diagnostic purposes requires much effort, it is quite possible for the individual skilled in the art to derive, from the selected DNA fragments, oligonucleotides other than the ones listed in Annexes I to III, XXI to XXII, XXXII to XXXVII, XXXIX to XLI, and XLIII to LIV which are suitable for diagnostic purposes. When a proprietary fragment or a public databases sequence is selected for its specificity and ubiquity, it increases the probability that subsets thereof will also be specific and ubiquitous.

Since a high percentage of clinical specimens are negative for bacteria (Table 3), DNA fragments having a high potential for the selection of universal oligonucleotide probes or primers were selected from proprietary and public database sequences. The amplification primers were selected from genes highly conserved in algae, archaea, bacteria, fungi and parasites, and are used to detect the presence of any algal, archaeal, bacterial, fungal or parasitical pathogen in clinical specimens in order to determine rapidly whether it is positive or negative for algae,

archaea, bacteria, fungi or parasites. The selected genes, designated *tuf, fus, atpD* and *recA,* encode respectively 2 proteins (elongation factors Tu and G) involved in the translational process during protein synthesis, a protein (beta subunit) responsible for the catalytic activity of proton pump ATPase and a protein responsible for the homologous recombination of genetic material. The alignments of *tuf, atpD* and *recA* sequences used to derive the universal primers include both proprietary and public database sequences. The universal primer strategy allows the rapid screening of the numerous negative clinical specimens (around 80% of the specimens received, see Table 3) submitted for microbiological testing.

Table 4 provides a list of the archaeal, bacterial, fungal and parasitical species for which *tuf* and/or *atpD* and/or *recA* nucleic acids and/or sequences are revealed in the present invention. Tables 5 and 6 provide a list of antimicrobial agents resistance genes and toxin genes selected for diagnostic purposes. Table 7 provides the origin of *tuf, atpD* and *recA* nucleic acids and/or sequences listed in the sequence listing. Tables 8-10 and 12-14 provide lists of species used to test the specificity, ubiquity and sensitivity of some assays described in the examples. Table 11 provides a list of microbial species for which *tuf* and/or *atpD* and/or *recA* sequences are available in public databases. Table 15 lists the microorganisms identified by commercial systems. Tables 16-18 are part of Example 42, whereas Tables 19-20 are part of Example 43. Tables 21-22 illustrate Example 44, whereas Tables 23-25 illustrate Example 45.

In accordance with the present invention is provided a method for generating a repertory of nucleic acids of *tuf*, *fus*, *atpD* and/or *recA* genes from which are derived probes or primers, or both, useful for the detection of one, more than one related microorganisms, or substantially all microorganisms of a group selected from algae, archaea, bacteria, fungi and parasites, which comprises the step of:

- amplifying the nucleic acids of a plurality of determined algal, archaeal, bacterial, fungal and parasitical species with any combination of the primer pairs defined in SEQ ID NOs.: 558-561, 562-574, 636-655, 664, 681-683, 696-697, 699-700, 708, 812-815, 911-917, 919-922, 935-938, 1203-1207, 1212-1213, 1221-1229, 1605-1606, 1974-1984, 1999- 2003, 2282-2285.

The terms "related microorganisms" are intended to cover microorganisms that share a common evolutive profile up to the speciation e.g. those that belong to a species, a genus, a family or a phyllum. The same terms are also intended to cover a group of different species that are grouped for a specific reason, for example, because they all have a common host tissue or cell. In one specific example, a group of microorganims potentially found in platelet preparations are grouped together and are considered "related" organisms for the purpose of their simultaneous detection in that particular type of sample.

The repertories *per se* of nucleic acids and of sequences derived therefrom are also provided, as well as "gene banks" comprising these repertories.

For generating sequences of probes or primers, the above method is reproduced or one may start from the sequence repertory or gene bank itself, and the following steps are added:

- aligning a subset of nucleic acid sequences of said repertory,
- locating nucleic acid stretches that are present in the nucleic acids of strains or representatives of said one, more than one related microorganisms, or substantially all microorganisms of said group, and not present in the nucleic acid sequences of other microorganisms, and

deriving consensus nucleic acid sequ nces useful as probes or primers from said stretches.

Once the sequences of probes or primers are designed, they are converted into real molecules by nucleic acid synthesis.

From the above methods and resulting repertories, probes and primers for the universal detection of any one of alga, archaeon, bacterium, fungus and parasite are obtainable.

More specifically, the following probes or primers having the sequence defined in SEQ ID NOs.: 543, 556-574, 636-655, 658-661, 664, 681-683, 694, 696, 697, 699, 700, 708, 812-815, 911-917, 919-922, 935-938, 1203-1207, 1212-1213, 1221-1229, 1605-1606, 1974-1984, 1999-2000, 2282-2285 or any variant of at least 12 nucleotides capable of hybridizing with the targeted microorganism(s) and these sequences and a diagnostic method using the same are provided.

Further, probes or primers having specific and ubiquitous properties for the detection and identification of any one of an algal, archaeal, bacterial, fungal and parasitital species, genus, family and group are also designed and derived from the same methods and repertories.

More specifically, are provided definite probes or primers having specific and ubiquitous properties for the detection and identification of microorganisms.

Indeed, a general method is provided for detecting the presence in a test sample of any microorganism that is an alga, archaeum, bacterium, fungus or parasite, which comprises:

a) putting in contact any test sample *tuf* or *atpD* or *recA* sequences and nucleic acid primers and/or probes, said primers and/or probes having be n selected to be sufficiently complementary to hybridize to

one or more *tuf* or *atpD* or *recA* sequences that are specific to said microorganism:

b) allowing the primers and/or probes and any test sample *tuf* or *atpD* or *recA* sequences to hybridize under specified conditions such as said primers and/or probes hybridize to the *tuf* or *atpD* or *recA* sequences of said microorganism and does not delectably hybridize to *tuf* or *atpD* or *recA* sequences from other microorganisms; and,

c) testing for hybridization of said primers and/or probes to any test sample *tuf* or *atpD* or *recA* sequences.

In the latter, step c) is based on a nucleic acid target amplification method, or on a signal amplification method.

The terms "sufficiently complementary" cover perfect and imperfect complementarity.

In addition to the universal or the specific detection and/or identification of microorganisms, the simultaneous detection of antimicrobial agent resistance gene or of a toxin gene is provided in compositions of matter as well as in diagnostic methods. Such detection is brought by using probes or primers having at least 12 nucleotides in length capable of hybridizing with an antimicrobial agent resistance gene and/or toxin gene, a definite set thereof being particularly provided.

Of course, any propriatory nucleic acid and nucleotide sequence derived therefrom, and any variant of at least 12 nucleotides capable of a selective hybridization with the following nucleic acids are within the scope of this invention as well as derived recombinant vectors and hosts:

SEQ ID NOs.: 1-73, 75-241, 399-457, 498-529, 612-618, 621-624, 675, 677, 717-736, 779-792, 840-855, 865, 868-888, 897-910, 932, 967-989 992, 1266-1297, 1518-1526, 1561-1575, 1578-1580, 1662-1664, 1666-1667, 1669-1670, 1673-1683, 1685-1689, 1786-1843, 1874-1881, 1956-1960, 2183-2185, 2187-2188, 2193-2201, 2214-2249, 2255-2272, which are all *tuf* sequences;

SEQ ID NOs.: 242-270, 272-398, 458-497, 530-538, 663, 667, 673-676, 678-680, 737-778, 827-832, 834-839, 856-862, 866-867, 889-896, 929-931, 941-966, 1245-1254, 1256-1265, 1527, 1576-1577, 1600-1604,1638-1647, 1649-1660, 1671, 1684, 1844-1848, 1849-1865, 2189-2192, which are all *atpD* sequences;

SEQ ID NOs.: 990-991, 1003, 1288-1289, 1714, 1756-1763, 1866-1873 and 2202-2212, which are all *recA* sequences; and

SEQ ID NOs.: 1004-1075, 1255, 1607-1608, 1648, 1764-1785, 2013-2014, 2056-2064, 2273-2280, which are antimicrobial agent resistance or toxin gene sequences found to be suitable for the detection and identification of microbial species.

To complement the following repertories, another one comprising *hexA* nucleic acids and derived sequences have been construed through amplification of nucleic acids of any streptococcal species with any combination of primers SEQ ID NOs.: 1179, 1181, 1182 and 1184 to 1191. From this particular repertory, primers and/or probes for detecting *Streptococcus pneumoniae* have been designed and obtained. Particularly, a nucleic acid sequence of at least 12 nucleotides capable of hybridizing with *Streptococcus pneumoniae* and with any one of SEQ ID NOs.: 1184 to 1187 or with SEQ ID NOs.: 1179, 1180, 1181 or 1182 are provided.

The remarkable sequence diversity of nucleic acids that encode proteins also provides diversity of peptide sequences which constitute another repertory that is also within the scope of this invention. From the protein and nucleic acid sequence repertories is derived a use therefrom for the design of a therapeutic agent effective against a target microorganism, for example, an antibiotic, a vaccine or a genic therapeutic agent.

Due to the constant evolution in the diagnostic methods, here is finally provided a method for the identification of a microorganism in a test sample, comprising the steps of:

a) obtaining a nucleic acid sequence from a *tuf*, *fus*, *atpD*, and/or *recA* genes of said microorganisms, and

b) comparing said nucleic acid sequence with the nucleic acid sequences of a bank as defined in claim 5, said repertory comprising a nucleic acid sequence obtained from the nucleic acids of said microorganism, whereby said microorganism is identify when there is a match between the sequences.

In this method, any way by which the specified given sequence is obtained is contemplated, and this sequence is simply compared to the sequences of a bank or a repertory. If the comparison results in a match, e.g. if bank comprises the nucleic acid sequence of interest, the identification of the microorganism is provided.

DETAILED DESCRIPTION OF THE INVENTION

HIGHLY CONSERVED GENES AND THEIR USE TO GENERATE SPECIES-SPECIFIC, GENUS-SPECIFIC, FAMILY-SPECIFIC, GROUP-SPECIFIC AND UNIVERSAL NUCLEIC ACID PROBES AND AMPLIFICATION PRIMERS TO RAPIDLY DETECT AND IDENTIFY ALGAL, ARCHAEAL, BACTERIAL, FUNGAL AND PARASITICAL MICROORGANISMS FROM CLINICAL SPECIMENS FOR DIAGNOSIS

The present inventors reasoned that comparing the published *Haemophilus influenzae* and *Mycoplasma genitalium* genomes and searching for conserved genes could provide targets to develop useful diagnostic primers and probes. This sequence comparison is highly informative as these two bacteria are distantly related and most genes present in the minimal genome of *M. genitalium* are likely to be present in every bacterium. Therefore genes conserved between these two bacteria are likely to be conserved in all other bacteria.

Following the genomic comparison, it was found that several protein-coding genes were conserved in evolution. Highly conserved proteins included the translation elongation factors G (EF-G) and Tu (EF-Tu) and the β subunit of F_0F_1 type ATP-synthase, and to a lesser extent, the RecA recombinase. These four proteins coding genes were selected amongst the 20 most conserved genes on the basis that they all possess at least two highly conserved regions suitable for the design of universal amplification and sequencing primers. Moreover, within the fragment amplified by these primers, highly conserved and more variable regions are also present hence suggesting it might be possible to rapidly obtain sequence information from various microbial species to design universal as well as species-, genus-, family-, or group-specific primers and probes of potential use for the detection and identification and/or quantification of microorganisms.

Translation elongation factors are members of a family of GTP-binding proteins which intervene in the interactions of tRNA molecules with the ribosome machinery during essential steps of protein synthesis. The role of elongation factor Tu is to facilitate the binding of aminoacylated tRNA molecules to the A site of the ribosome. The eukaryotic, archaeal (archaebacterial) and algal homolog of EF-Tu is called elongation factor 1 alpha (EF-1α). All protein synthesis factors originated from a common ancestor via gene duplications and fusions (Cousineau *et al.*, 1997, J. Mol. Evol. **45**:661-670). In particular, elongation factor G (EF-G), although having a functional role in promoting the translocation of aminoacyl-tRNA molecules from the A site to the P site of the ribosome, shares sequence homologies with EF-Tu and is thought to have arisen from the duplication and fusion of an ancestor of the EF-Tu gene.

In addition, EF-Tu is known to be the target for antibiotics belonging to the elfamycin's group as well as to other structural classes (Anborgh and Parmeggiani, 1991, EMBO J. **10**:779-784; Luiten *et al.*, 1992, European patent application serial No. EP 0 466 251 A1). EF-G for its part, is the target of the antibiotic fusidic acid. In addition to its crucial activities in translation, EF-Tu has chaperone-like functions in protein folding, protection against heat denaturation of proteins and interactions with unfolded proteins (Caldas *et al.*, 1998, J. Biol. Chem **273**:11478-11482). Interestingly, a form of the EF-Tu protein has been identified as a dominant component of the periplasm of *Neisseria gonorrhoeae* (Porcella *et al.*, 1996, Microbiology **142**:2481-2489), hence suggesting that at least in some bacterial species, EF-Tu might be an antigen with vaccine potential.

F_0F_1 type ATP-synthase belongs to a superfamily of proton-translocating ATPases divided in three major families: P, V and F (Nelson and Taiz, 1989, TIBS **14**:113-116). P-ATPases (or E_1-E_2 type) operate via a phosphorylated intermediate and are not evolutionarily related to the other two families. V-ATPases (or V_0V_1 type) are present on the vacuolar and other endomembranes of eukaryotes, on the plasma membrane of archaea (archaebacteria) and algae, and also on the plasma membrane of some eubacteria especially species belonging to the order

Spirochaetales as well as to the *Chlamydiaceae* and *Deinococcaceae* families. F-ATPases (or F_0F_1 type) are found on the plasma membrane of most eubacteria, on the inner membrane of mitochondria and on the thylakoid membrane of chloroplasts. They function mainly in ATP synthesis. They are large multimeric enzymes sharing numerous structural and functional features with the V-ATPases. F and V-type ATPases have diverged from a common ancestor in an event preceding the appearance of eukaryotes. The β subunit of the F-ATPases is the catalytic subunit and it possesses low but significant sequence homologies with the catalytic A subunit of V-ATPases.

The translation elongation factors EF-Tu, EF-G and EF-1α, and the catalytic subunit of F or V-types ATP-synthase, are highly conserved proteins sometimes used for phylogenetic analysis and their genes are also known to be highly conserved (Iwabe *et al.*, 1989, Proc. Natl. Acad. Sci. USA **86**:9355-9359, Gogarten *et al.*, 1989, Proc. Natl. Acad. Sci. USA **86**:6661-6665, Ludwig *et al.*, 1993, Antonie van Leeuwenhoek **64**:285-305). A recent BLAST (Altschul *et al.*, 1997, J. Mol. Biol. **215**:403-410) search performed by the present inventors on the GenBank, European Molecular Biology Laboratory (EMBL), DNA Database of Japan (DDBJ) and specific genome project databases indicated that throughout bacteria, the EF-Tu and the β subunit of F_0F_1 type ATP-synthase genes may be more conserved than other genes that are well conserved between *H. influenzae* and *M. genitalium*.

The RecA recombinase is a multifunctional protein encoded by the *recA* gene. It plays a central role in homologous recombination, it is critical for the repair of DNA damage and it is involved in the regulation of the SOS system by promoting the proteolytic digestion of the LexA repressor. It is highly conserved in bacteria and could serve as a useful genetic marker to reconstruct bacterial phylogeny (Miller and Kokjohn, 1990, Annu. Rev. Microbiol. **44**:365-394). Although RecA possesses some highly conserved sequence segments that we used to design universal primers aimed at sequencing the *recA* fragments, it is clearly not as well conserved EF-G, EF-Tu and β subunit of F_0F_1 type ATP-synthase.

Hence, RecA may not be optimal for universal detection of bacteria with high sensitivity but it was chosen because preliminary data indicated that EF-G, EF-Tu and β subunit of F_0F_1 type ATP-synthase may sometimes be too closely related to find specific primer pairs that could discriminate between certain very closely related species and genera. While RecA, EF-G, EF-Tu and β subunit of F_0F_1 type ATP-synthase genes, possesses highly conserved regions suitable for the design of universal sequencing primers, the less conserved region between primers should be divergent enough to allow species-specific and genus-specific primers in those cases.

Thus, as targets to design primers and probes for the genetic detection of microorganisms, the present inventors have focused on the genes encoding these four proteins: *tuf*, the gene for elongation factor Tu (EF-Tu); *fus*, the gene for the elongation factor G (EF-G); *atpD*, the gene for β subunit of F_0F_1 type ATP-synthase; and *recA*, the gene encoding the RecA recombinase. In several bacterial genomes *tuf* is often found in two highly similar duplicated copies named *tufA* and *tufB* (Filer and Furano, 1981, J. Bacteriol. **148**:1006-1011, Sela *et al.*, 1989, J. Bacteriol. **171**:581-584). In some particular cases, more divergent copies of the *tuf* genes can exist in some bacterial species such as some actinomycetes (Luiten *et al.* European patent application publication No. EP 0 446 251 A1; Vijgenboom *et al.*, 1994, Microbiology **140**:983-998) and, as revealed as part of this invention, in several enterococcal species. In several bacterial species, *tuf* is organized in an operon with its homolog gene for the elongation factor G (EF-G) encoded by the *fusA* gene (Figure 3). This operon is often named the *str* operon. The *tuf*, *fus*, *atpD* and *recA* genes were chosen as they are well conserved in evolution and have highly conserved stretches as well as more variable segments. Moreover, these four genes have eukaryotic orthologs which are described in the present invention as targets to identify fungi and parasites. The eukaryotic homolog of elongation factor Tu is called elongation factor 1-alpha (EF-1α) (gene name: *tef*, *tef1*, *ef1*, *ef-1* or *EF-1*). In fungi, the gene for EF-1α occurs sometimes in two or more highly

similar duplicated copies (often named *tef1*, *tef2*, *tef3*...). In addition, eukaryotes have a copy of elongation factor Tu which is originating from their organelle genome ancestry (gene name: *tuf1*, *tufM* or *tufA*). For the purpose of the current invention, the genes for these four functionally and evolutionarily linked elongation factors (bacterial EF-Tu and EF-G, eukaryotic EF-1α, and organellar EF-Tu) will hereafter be designated as «*tuf* nucleic acids and/or sequences». The eukaryotic (mitochondrial) F_0F_1 type ATP-synthase beta subunit gene is named *atp2* in yeast. For the purpose of the current invention, the genes of catalytic subunit of either F or V-type ATP-synthase will hereafter be designated as «*atpD* nucleic acids and/or sequences». The eukaryotic homologs of RecA are distributed in two families, typified by the Rad51 and Dmc1 proteins. Archaeal homologs of RecA are called RadA. For the purpose of the current invention, the genes corresponding to the latter proteins will hereafter be designated as «*recA* nucleic acids and/or sequences».

In the description of this invention, the terms «nucleic acids» and «sequences» might be used interchangeably. However, «nucleic acids» are chemical entities while «sequences» are the pieces of information derived from (inherent to) these «nucleic acids». Both nucleic acids and sequences are equivalently valuable sources of information for the matter pertaining to this invention.

Analysis of multiple sequence alignments of *tuf* and *atpD* sequences permitted the design of oligonucleotide primers (and probes) capable of amplifying (or hybridizing to) segments of *tuf* (and/or *fus*) and *atpD* genes from a wide variety of bacterial species (see Examples 1 to 4, 24 and 26, and Table 7). Sequencing and amplification primer pairs for *tuf* nucleic acids and/or sequences are listed in Annex I and hybridization probes are listed in Annexes III and XLVII. Sequencing and amplification primer pairs for *atpD* nucleic acids and/or sequences are listed in Annex II. Analysis of the main subdivisions of *tuf* and *atpD* sequences (see Figures 1 and 2) permitted to design sequencing primers amplifying specifically each of these subdivisions. It should be noted that these sequencing primers could also be used as universal primers. However, since some of these sequencing primers

include several variable sequence (degenerated) positions, their sensitivity could be lower than that of universal primers developed for diagnostic purposes. Further subdivisions could be done on the basis of the various phyla where these genes are encountered.

Similarly, analysis of multiple sequence alignments of *recA* sequences present in the public databases permitted the design of oligonucleotide primers capable of amplifying segments of *recA* genes from a wide variety of bacterial species. Sequencing and amplification primer pairs for *recA* sequences are listed in Annex XXI. The main subdivisions of *recA* nucleic acids and/or sequences comprise *recA*, *radA*, *rad51* and *dmc1*. Further subdivisions could be done on the basis of the various phyla where these genes are encountered.

The present inventor's strategy is to get as much sequence data information from the four conserved genes (*tuf*, *fus*, *atpD* and *recA*). This ensemble of sequence data forming a repertory (with subrepertories corresponding to each target gene and their main sequence subdivisions) and then using the sequence information of the sequence repertory (or subrepertories) to design primer pairs that could permit either universal detection of algae or archaea or bacteria or fungi or parasites, detection of a family or group of microorganism (e.g. *Enterobacteriaceae*), detection of a genus (e.g. *Streptococcus*) or finally a specific species (e.g. *Staphylococcus aureus*). It should be noted that for the purpose of the present invention a group of microorganisms is defined depending on the needs of the particular diagnostic test. It does not need to respect a particular taxonomical grouping or phylum. See Example 12 where primers were designed to amplify a group a bacteria consisting of the 17 major bacterial species encountered as contaminants of platelet concentrates. Also remark that in that Example, the primers are not only able to sensitively and rapidly detect at least the 17 important bacterial species, but could also detect other species as well, as shown in Table 14. In these circumstances the primers shown in Example 12 are considered universal for platelet-contaminating bacteria. To develop an assay specific for the latter, one or more primers or probes specific to each species could be designed. Another

example of primers and/or probes for group detection is given by the Pseudomonad group primers. These primers were designed based upon alignment of *tuf* sequences from real *Pseudomonas* species as well as from former *Pseudomonas* species such as *Stenotrophomonas maltophilia*. The resulting primers are able to amplify all *Pseudomonas* species tested as well as several species belonging to different genera, hence as being specific for a group including *Pseudomonas* and other species, we defined that group as Pseudomonads, as several members were former *Pseudomonas*.

For certain applications, it may be possible to develop a universal, group, family or genus-specific reaction and to proceed to species identification using sequence information within the amplicon to design species-specific internal probes or primers, or alternatively, to proceed directly by sequencing the amplicon. The various strategies will be discussed further below.

The ensembles formed by public and proprietary *tuf, atpD* and *rec*A nucleic acids and/or sequences are used in a novel fashion so they constitute three databases containing useful information for the identification of microorganisms.

Sequence repertories of other gene targets were also built to solve some specific identification problems especially for microbial species genetically very similar to each other such as *E. coli* and *Shigella* (see Example 23). Based on *tuf, atpD* and *rec*A sequences, *Streptococcus pneumoniae* is very difficult to differentiate from the closely related species *S. oralis* and *S. mitis*. Therefore, we elected to built a sequence repertory from *hexA* sequences (Example 19), a gene much more variable than our highly conserved *tuf* , *atpD* and *recA* nucleic acids and/or sequences.

For the detection of mutations associated with antibiotic resistance genes, we also built repertories to distinguish between point mutations reflecting only gene diversity and point mutations involved in resistance. This was done for *pbp1a, pbp2b* and *pbp2x* genes of penicillin-resistant and sensitive *Streptoccoccus pneumoniae* (Example 18) and also for *gyrA* and *parC* gene fragments of various bacterial species for which quinolone resistance is important to monitor.

Oligonucleotide primers and probes design and synthesis

The *tuf, fus, atpD* and *recA* DNA fragments sequenced by us and/or selected from public databases (GenBank and EMBL) were used to design oligonucleotides primers and probes for diagnostic purposes. Multiple sequence alignments were made using subsets of the *tuf* or *atpD* or *recA* sequences repertory. Subsets were chosen to encompass as much as possible of the targetted microorganism(s) DNA sequence data and also include sequence data from phylogenetically related microorganisms from which the targetted microorganism(s) should be distinguished. Regions suitable for primers and probes should be conserved for the targetted microorganism(s) and divergent for the microorganisms from which the targetted microorganism(s) should be distinguished. The large amount of *tuf* or *atpD* or *recA* sequences data in our repertory permits to reduce trial and errors in obtaining specific and ubiquitous primers and probes. We also relied on the corresponding peptide sequences of *tuf, fus, atpD* and *recA* nucleic acids and/or sequences to facilitate the identification of regions suitable for primers and probes design. As part of the design rules, all oligonucleotides (probes for hybridization and primers for DNA amplification by PCR) were evaluated for their suitability for hybridization or PCR amplification by computer analysis using standard programs (i.e. the Genetics Computer Group (GCG) programs and the primer analysis software Oligo™ 5.0). The potential suitability of the PCR primer pairs was also evaluated prior to the synthesis by verifying the absence of unwanted features such as long stretches of one nucleotide and a high proportion of G or C residues at the 3' end (Persing *et al.*, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.). Oligonucleotide probes and amplification primers were synthesized using an automated DNA synthesizer (Perkin-Elmer Corp., Applied Biosystems Division).

The oligonucleotide sequence of primers or probes may be derived from either strand of the duplex DNA. The primers or probes may consist of the bases

A, G, C, or T or analogs and they may be degenerated at one or more chosen nucleotide position(s). The primers or probes may be of any suitable length and may be selected anywhere within the DNA sequences from proprietary fragments or from selected database sequences which are suitable for (i) the universal detection of algae or archaea or bacteria or fungi or parasites, (ii) the species-specific detection and identification of any microorganism, including but not limited to: *Abiotrophia adiacens*, *Bacteroides fragilis*, *Bordetella pertussis*, *Candida albicans*, *Candida dubliniensis*, *Candida glabrata*, *Candida guilliermondii*, *Candida krusei*, *Candida lusitaniae*, *Candida parapsilosis*, *Candida tropicalis*, *Candida zeylanoides*, *Campylobacter jejuni and C. coli*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Cryptococcus neoformans*, *Cryptosporidium parvum*, *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus gallinarum*, *Escherichia coli*, *Haemophilus influenzae*, *Legionella pneumophila*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus saprophyticus*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Trypanosoma brucei*, *Trypanosoma cruzi*, (iii) the genus-specific detection of *Bordetella* species, *Candida* species, *Clostridium* species, *Corynebacterium* species, *Cryptococcus* species, *Entamoeba* species, *Enterococcus* species, *Gemella* species, *Giardia* species, *Legionella* species, *Leishmania* species, *Staphylococcus* species, *Streptococcus* species, *Trypanosoma* species, (iv) the family-specific detection of *Enterobacteriaceae* family members, *Mycobacteriaceae* family members, *Trypanosomatidae* family members, (v) the detection of *Enterococcus casseliflavus-flavescens-gallinarum* group, *Enterococcus*, *Gemella* and *Abiotrophia adiacens* group, Pseudomonads extended group, Platelet-contaminating bacteria group, (vi) the detection of clinically important antimicrobial agents resistance genes listed in Table 5, (vii) the detection of clinically important toxin genes listed in Table 6.

Variants for a given target microbial gene are naturally occurring and are attributable to sequence variation within that gene during evolution (Watson *et al.*, 1987, Molecular Biology of the Gene, 4th ed., The Benjamin/Cummings Publishing Company, Menlo Park, CA; Lewin, 1989, Genes IV, John Wiley & Sons, New York, NY). For example, different strains of the same microbial species may have a single or more nucleotide variation(s) at the oligonucleotide hybridization site. The person skilled in the art is well aware of the existence of variant algal, archaeal, bacterial, fungal or parasitical DNA nucleic acids and/or sequences for a specific gene and that the frequency of sequence variations depends on the selective pressure during evolution on a given gene product. The detection of a variant sequence for a region between two PCR primers may be demonstrated by sequencing the amplification product. In order to show the presence of sequence variants at the primer hybridization site, one has to amplify a larger DNA target with PCR primers outside that hybridization site. Sequencing of this larger fragment will allow the detection of sequence variation at this site. A similar strategy may be applied to show variants at the hybridization site of a probe. Insofar as the divergence of the target nucleic acids and/or sequences or a part thereof does not affect the specificity and ubiquity of the amplification primers or probes, variant microbial DNA is under the scope of this invention. Variants of the selected primers or probes may also be used to amplify or hybridize to a variant DNA.

Sequencing of *tuf* nucleic acids and/or sequences from a variety of archaeal, bacterial, fungal and parasitical species

The nucleotide sequence of a portion of *tuf* nucleic acids and/or sequences was determined for a variety of archaeal, bacterial, fungal and parasitical species. The amplification primers (SEQ ID NOs. 664 and 697), which amplify a *tuf* gene portion of approximately 890 bp, were used along with newly designed sequencing primer pairs (See Annex I for the sequencing primers for *tuf* nucleic acids and/or

sequences). Most primer pairs can amplify different copies of *tuf* genes (*tufA* and *tufB*). This is not surprising since it is known that for several bacterial species these two genes are nearly identical. For example, the entire *tufA* and *tufB* genes from *E. coli* differ at only 13 nucleotide positions (Neidhardt *et al.*, 1996, *Escherichia coli* and *Salmonella*: Cellular and Molecular Biology, 2[nd] ed., American Society for Microbiology Press, Washington, D.C.). Similarly, some fungi are known to have two nearly identical copies of *tuf* nucleic acids and/or sequences (EF-1α). These amplification primers are degenerated at several nucleotide positions and contain inosines in order to allow the amplification of a wide range of *tuf* nucleic acids and/or sequences. The strategy used to select these amplification primers is similar to that illustrated in Annex I for the selection of universal primers. The *tuf* sequencing primers even sometimes amplified highly divergent copies of *tuf* genes (*tufC*) as illustrated in the case of some enterococcal species (SEQ ID NOs.: 73, 75, 76, 614 to 618, 621 and 987 to 989). To prove this, we have determined the enterococcal *tuf* nucleic acids and/or sequences from PCR amplicons cloned into a plasmid vector. Using the sequence data from the cloned amplicons, we designed new sequencing primers specific to the divergent (*tufC*) copy of enterococci(SEQ ID NOs.: 658-659 and 661) and then sequenced directly the *tufC* amplicons. The amplification primers (SEQ ID NOs.: 543, 556, 557, 643-645, 660, 664, 694, 696 and 697) could be used to amplify the *tuf* nucleic acids and/or sequences from any bacterial species. The amplification primers (SEQ ID NOs.: 558, 559, 560, 653, 654, 655, 813, 815, 1974-1984, 1999-2003) could be used to amplify the *tuf* (EF-1α) genes from any fungal and/or parasitical species. The amplification primers SEQ ID NOs. 1221-1228 could be used to amplify bacterial *tuf* nucleic acids and/or sequences of the EF-G subdivision (*fusA*) (Figure 3). The amplification primers SEQ ID NOs. 1224, and 1227-1229 could be used to amplify bacterial *tuf* nucleic acids and/or sequences comprising the end of EF-G (*fusA*) and the beginning of EF-Tu (*tuf*), including the intergenic region, as shown in Figure 3.

Most *tuf* fragments to be sequenced were amplified using the following amplification protocol: One μl of cell suspension (or of purified genomic DNA

0.1-100 ng/μl) was transferred directly to 19 μl of a PCR reaction mixture. Each PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM $MgCl_2$, 1 μM of each of the 2 primers, 200 μM of each of the four dNTPs, 0.5 unit of *Taq* DNA polymerase (Promega Corp., Madison, WI). PCR reactions were subjected to cycling using a PTC-200 thermal cycler (MJ Research Inc., Watertown, Mass.) as follows: 3 min at 94-96 °C followed by 30-45 cycles of 1 min at 95 °C for the denaturation step, 1 min at 50-55 °C for the annealing step and 1 min at 72 °C for the extension step. Subsequently, twenty microliters of the PCR-amplified mixture were resolved by electrophoresis in a 1.5% agarose gel. The amplicons were then visualized by staining with methylene blue (Flores *et al.*, 1992, Biotechniques, **13**:203-205). The size of the amplification products was estimated by comparison with a 100-bp molecular weight ladder. The band corresponding to the specific amplification product was excised from the agarose gel and purified using the QIAquick™ gel extraction kit (QIAGEN Inc., Chatsworth, CA). The gel-purified DNA fragment was then used directly in the sequencing protocol. Both strands of the *tuf* genes amplification product were sequenced by the dideoxynucleotide chain termination sequencing method by using an Applied Biosystems automated DNA sequencer (model 377) with their Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA). The sequencing reactions were performed by using the same amplification primers and 10 ng/100 bp of the gel-purified amplicon per reaction. For the sequencing of long amplicons such as those of eukaryotic *tuf* (EF-1α) nucleic acids and/or sequences, we designed internal sequencing primers (SEQ ID NOs.: 654, 655 and 813) to be able to obtain sequence data on both strands for most of the fragment length. In order to ensure that the determined sequence did not contain errors attributable to the sequencing of PCR artefacts, we have sequenced two preparations of the gel-purified *tuf* amplification product originating from two independent PCR amplifications. For most target microbial species, the sequences determined for both amplicon preparations were identical. In case of discrepancies, amplicons from a third independent PCR amplification

were sequenced. Furthermore, the sequences of both strands were 100% complementary thereby confirming the high accuracy of the determined sequence. The *tuf* nucleic acids and/or sequences determined using the above strategy are described in the Sequence Listing. Table 7 gives the originating microbial species and the source for each *tuf* sequence in the Sequence Listing.

The alignment of the *tuf* sequences determined by us or selected from databases revealed clearly that the length of the sequenced portion of the *tuf* genes is variable. There may be insertions or deletions of several amino acids. In addition, in several fungi introns were observed. Intron nucleic acids and/or sequences are part of *tuf* nucleic acids and/or sequences and could be useful in the design of species-specific primers and probes. This explains why the size of the sequenced *tuf* amplification products was variable from one fungal species to another. Consequently, the nucleotide positions indicated on top of each of Annexes IV to XX, XXIII to XXXI, XXXVIII and XLII do not correspond for sequences having insertions or deletions.

It should also be noted that the various *tuf* nucleic acids and/or sequences determined by us occasionally contain base ambiguities. These degenerated nucleotides correspond to sequence variations between *tufA* and *tufB* genes (or copies of the EF-G subdivision of *tuf* nucleic acids and/or sequences, or copies of EF-1α subdivision of *tuf* nucleic acids and/or sequences for fungi and parasites) because the amplification primers amplify both *tuf* genes. These nucleotide variations were not attributable to nucleotide misincorporations by the *Taq* DNA polymerase because the sequence of both strands was identical and also because the sequences determined with both preparations of the gel-purified *tuf* amplicons obtained from two independent PCR amplifications were identical.

<u>The selection of amplification primers from *tuf* nucleic acids and/or sequences</u>

The *tuf* sequences determined by us or selected from public databases were used to select PCR primers for universal detection of bacteria, as well as for genus-

specific, species-specific family-specific or group-specific detection and identification. The strategy used to select these PCR primers was based on the analysis of multiple sequence alignments of various *tuf* sequences. For more details about the selection of PCR primers from *tuf* sequences please refer to Examples 5, 7-14, 17, 22, 24, 28, 30-31, 33, 36, and 38-40, and to Annexes VI-IX, XI-XIX and XXV.

Sequencing of *atpD* and *recA* nucleic acids and/or sequences from a variety of archaeal, bacterial, fungal and parasitical species

The method used to obtain *atpD* and *recA* nucleic acids and/or sequences is similar to that described above for *tuf* nucleic acids and/or sequences.

The selection of amplification primers from *atpD* or *recA* nucleic acids and/or sequences

The comparison of the nucleotide sequence for the *atp*D or *rec*A genes from various archaeal, bacterial, fungal and parasitical species allowed the selection of PCR primers (refer to Examples 6, 13, 29, 34 and 37, and to Annexes IV, V, X, and XX).

DNA amplification

For DNA amplification by the widely used PCR (polymerase chain reaction) method, primer pairs were derived from proprietary DNA fragments or from database sequences. Prior to synthesis, the potential primer pairs were analyzed by using the Oligo™ 5.0 software to verify that they were good candidates for PCR amplification.

During DNA amplification by PCR, two oligonucleotide primers binding respectively to each strand of the heat-denatured target DNA from the microbial

genome are used to amplify exponentially *in vitro* the target DNA by successive thermal cycles allowing denaturation of the DNA, annealing of the primers and synthesis of new targets at each cycle (Persing *et al*, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.).

Briefly, the PCR protocols were as follows: Treated clinical specimens or standardized bacterial or fungal or parasitical suspensions (see below) or purified genomic DNA from bacteria, fungi or parasites were amplified in a 20 μl PCR reaction mixture. Each PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 2.5 mM $MgCl_2$, 0.4 μM of each primer, 200 μM of each of the four dNTPs and 0.5 unit of *Taq* DNA polymerase (Promega) combined with the TaqStart™antibody (Clontech Laboratories Inc., Palo Alto, CA). The TaqStart™ antibody, which is a neutralizing monoclonal antibody to *Taq* DNA polymerase, was added to all PCR reactions to enhance the specificity and the sensitivity of the amplifications (Kellogg *et al*., 1994, Biotechniques **16**:1134-1137). The treatment of the clinical specimens varies with the type of specimen tested, since the composition and the sensitivity level required are different for each specimen type. It consists in a rapid protocol to lyse the microbial cells and eliminate or neutralize PCR inhibitors. For amplification from bacterial or fungal or parasitical cultures or from purified genomic DNA, the samples were added directly to the PCR amplification mixture without any pre-treatment step. An internal control was derived from sequences not found in the target microorganisms or in the human genome. The internal control was integrated into all amplification reactions to verify the efficiency of the PCR assays and to ensure that significant PCR inhibition was absent. Alternatively, an internal control derived from rRNA was also useful to monitor the efficiency of microbial lysis protocols.

PCR reactions were then subjected to thermal cycling (3 min at 94-96°C followed by 30 cycles of 1 second at 95°C for the denaturation step and 30 seconds at 50-65°C for the annealing-extension step) using a PTC-200 thermal cycler (MJ Research Inc.). The number of cycles performed for the PCR assays varies

according to the sensitivity level required. For example, the sensitivity level required for microbial detection directly from clinical specimens is higher for blood specimens than for urine specimens because the concentration of microorganisms associated with a septicemia can be much lower than that associated with a urinary tract infection. Consequently, more sensitive PCR assays having more thermal cycles are probably required for direct detection from blood specimens. Similarly, PCR assays performed directly from bacterial or fungal or parasitical cultures may be less sensitive than PCR assays performed directly from clinical specimens because the number of target organisms is normally much lower in clinical specimens than in microbial cultures.

The person skilled in the art of DNA amplification knows the existence of other rapid amplification procedures such as ligase chain reaction (LCR), transcription-mediated amplification (TMA), self-sustained sequence replication (3SR), nucleic acid sequence-based amplification (NASBA), strand displacement amplification (SDA), branched DNA (bDNA), cycling probe technology (CPT), solid phase amplification (SPA), rolling circle amplification technology (RCA), solid phase RCA, anchored SDA and nuclease dependent signal amplification (NDSA) (Lee *et al.*, 1997, Nucleic Acid Amplification Technologies: Application to Disease Diagnosis, Eaton Publishing, Boston, MA; Persing *et al.*, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.; Westin *et al.*, 2000, Nat. Biotechnol. 18:199-204). The scope of this invention is not limited to the use of amplification by PCR, but rather includes the use of any rapid nucleic acid amplification method or any other procedure which may be used to increase the sensitivity and/or the rapidity of nucleic acid-based diagnostic tests. The scope of the present invention also covers the use of any nucleic acids amplification and detection technology including real-time or post-amplification detection technologies, any amplification technology combined with detection, any hybridization nucleic acid chips or arrays technologies, any amplification chips or combination of amplification and

hybridization chips technologies. Detection and identification by any sequencing method is also under the scope of the present invention.

Any oligonucleotide suitable for the amplification of nucleic acids by approaches other than PCR or for DNA hybridization which are derived from the species-specific, genus-specific and universal DNA fragments as well as from selected antimicrobial agents resistance or toxin gene sequences included in this document are also under the scope of this invention.

Detection of amplification products

Classically, detection of amplification is performed by standard ethidium bromide-stained agarose gel electrophoresis. It is clear that other methods for the detection of specific amplification products, which may be faster and more practical for routine diagnosis, may be used. Such methods may be based on the detection of fluorescence after or during amplification. One simple method for monitoring amplified DNA is to measure its rate of formation by measuring the increase in fluorescence of intercalating agents such as ethidium bromide or SYBR® Green I (Molecular Probes). If more specific detection is required, fluorescence-based technologies can monitor the appearance of a specific product during the reaction. The use of dual-labeled fluorogenic probes such as in the TaqMan™ system (Applied Biosystems) which utilizes the 5'-3' exonuclease activity of the *Taq* polymerase is a good example (Livak K.J. *et al.* 1995, PCR Methods Appl. **4**:357-362). TaqMan™ can be performed during amplification and this "real-time" detection can be done in a single closed tube hence eliminating post-PCR sample handling and consequently preventing the risk of amplicon carryover. Several other fluorescence-based detection methods can be performed in real-time. Fluorescence resonance energy transfer (FRET) is the principle behind the use of adjacent hybridization probes (Wittwer, C.T. *et al.* 1997. BioTechniques **22**:130-138), molecular beacons (Tyagi S. and Kramer F.R. 1996. Nature Biotechnology **14**:303-308) and scorpions (Whitcomb *et al.* 1999. Nature

Biotechnology **17**:804-807). Adjacent hybridization probes are designed to be internal to the amplification primers. The 3' end of one probe is labelled with a donor fluorophore while the 5' end of an adjacent probe is labelled with an acceptor fluorophore. When the two probes are specifically hybridized in closed proximity (spaced by 1 to 5 nucleotides) the donor fluorophore which has been excited by an external light source emits light that is absorbed by a second acceptor that emit more fluorescence and yields a FRET signal. Molecular beacons possess a stem-and-loop structure where the loop is the probe and at the bottom of the stem a fluorescent moiety is at one end while a quenching moiety is at the other end. The beacons undergo a fluorogenic conformational change when they hybridize to their targets hence separating the fluorochrome from its quencher. The FRET principle is also used in an air thermal cycler with a built-in fluorometer (Wittwer, C.T. *et al.* 1997. BioTechniques **22**:130-138). The amplification and detection are extremely rapid as reactions are performed in capillaries: it takes only 18 min to complete 45 cycles. Those techniques are suitable especially in the case where few pathogens are searched for. Boehringer-Roche Inc. sells the LightCycler™, and Cepheid makes the SmartCycler. These two apparatus are capable of rapid cycle PCR combined with fluorescent SYBR® Green I or FRET detection. We recently demonstrated in our laboratory, real-time detection of 10 CFU in less than 40 minutes using adjacent hybridization probes on the LightCycler™. Methods based on the detection of fluorescence are particularly promising for utilization in routine diagnosis as they are very rapid, quantitative and can be automated.

Microbial pathogens detection and identification may also be performed by solid support or liquid hybridization using species-specific internal DNA probes hybridizing to an amplification product. Such probes may be generated from any sequence from our repertory and designed to specifically hybridize to DNA amplification products which are objects of the present invention. Alternatively, the internal probes for species or genus or family or group detection and identification may be derived from the amplicons produced by a universal, family-, group-, genus- or species-specific amplification assay(s). The oligonucleotide

probes may be labeled with biotin or with digoxigenin or with any other reporter molecule (for more details see below the section on hybrid capture). Hybrization on a solid support is amendable to miniaturization.

At present the oligonucleotide nucleic acid microarray technology is appealing. Currently, available low to medium density arrays (Heller *et al.*, An integrated microelectronics hybridization system for genomic research and diagnostic applications. *In*: Harrison, D.J., and van den Berg, A., 1998, Micro total analysis systems '98, Kluwer Academic Publisher, Dordrecht.) could specifically capture fluorescent-labelled amplicons. Detection methods for hybridization are not limited to fluorescence; potentiometry, colorimetry and plasmon resonance are some examples of alternative detection methods. In addition to detection by hybridization, nucleic acid microarrays could be used to perform rapid sequencing by hybridization. Mass spectrometry could also be applicable for rapid identification of the amplicon or even for sequencing of the amplification products (Chiu and Cantor, 1999, Clinical Chemistry **45**:1578; Berkenkamp *et al.*, 1998, Science **281**:260).

For the future of our assay format, we also consider the major challenge of molecular diagnostics tools, *i.e.*: integration of the major steps including sample preparation, genetic amplification, detection, data analysis and presentation (Anderson *et al.*, Advances in integrated genetic analysis. *In*: Harrison, D.J., and van den Berg, A., 1998, Micro total analysis systems '98, Kluwer Academic Publisher, Dordrecht.).

To ensure PCR efficiency, glycerol, dimethyl sulfoxide (DMSO) or other related solvents can be used to increase the sensitivity of the PCR and to overcome problems associated with the amplification of a target DNA having a high GC content or forming strong secondary structures (Dieffenbach and Dveksler, 1995, PCR Primer: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Plainview, New York). The concentration ranges for glycerol and DMSO are 5-15% (v/v) and 3-10% (v/v), respectively. For the PCR reaction mixture, the concentration ranges for the amplification primers and $MgCl_2$ are 0.1-1.5 μM and

1.0-10.0 mM, respectively. Modifications of the standard PCR protocol using external and nested primers (i.e. nested PCR) or using more than one primer pair (i.e. multiplex PCR) may also be used (Persing *et al.*, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.). For more details about the PCR protocols and amplicon detection methods, see Examples.

Hybrid capture and chemiluminescence detection of amplification products

Hybridization and detection of amplicons by chemiluminescence were adapted from Nikiforov *et al.* (1994, PCR Methods and Applications **3**:285-291 and 1995, Anal. Biochem. **227**:201-209) and from the DIG™ system protocol of Boehringer Mannheim. Briefly, 50 μl of a 25 picomoles solution of capture probe diluted in EDC {1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride} are immobilized in each well of 96-wells plates (Microlite™ 2, Dynex) by incubation overnight at room temperature. The next day, the plates are incubated with a solution of 1% BSA diluted into TNTw (10 mM Tris-HCl, pH 7.5; 150 mM NaCl; 0.05% Tween™ 20) for 1 hour at 37 °C. The plates are then washed on a Wellwash Ascent™ (Labsystems) with TNTw followed by Washing Buffer (100 mM maleic acid pH7.5; 150 mM NaCl; 0.3% Tween™ 20).

The amplicons were labelled with DIG-11-dUTP during PCR using the PCR DIG Labelling Mix from Boehringer Mannheim according to the manufacturer's instructions. Hybridization of the amplicons to the capture probes is performed in triplicate at stringent temperature (generally, probes are designed to allow hybrization at 55 °C, the stringent temperature) for 30 minutes in 1.5 M NaCl; 10 mM EDTA. It is followed by two washes in 2 X SSC; 0.1% SDS, then by four washes in 0.1X SSC; 0.1% SDS at the stringent temperature (55 °C). Detection with 1,2 dioxetane chemiluminescent alkaline phosphatase substrates like CSPD® (Tropix Inc.) is performed according to the manufacturer's instructions but with shorter incubations times and a different antibody concentration. The plates are

agitated at each step, the blocking incubation is performed for only 5 minutes, the anti-DIG-AP1 is used at a 1:1000 dilution, the incubation with antibody lasts 15 minutes, the plates are washed twice for only 5 minutes. Finally, after a 2 minutes incubation into the detection buffer, the plates are incubated 5 minutes with CSPD® at room temperature followed by a 10 minutes incubation at 37 °C without agitation. Luminous signal detection is performed on a Dynex Microtiter Plate Luminometer using RLU (Relative Light Units).

Specificity, ubiquity and sensitivity tests for oligonucleotide primers and probes

The specificity of oligonucleotide primers and probes was tested by amplification of DNA or by hybridization with bacterial or fungal or parasitical species selected from a panel comprising closely related species and species sharing the same anatomo-pathological site (see Annexes and Examples). All of the bacterial, fungal and parasitical species tested were likely to be pathogens associated with infections or potential contaminants which can be isolated from clinical specimens. Each target DNA could be released from microbial cells using standard chemical and/or physical treatments to lyse the cells (Sambrook *et al.*, 1989, Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY) or alternatively, genomic DNA purified with the GNOME™ DNA kit (Bio101, Vista, CA) was used. Subsequently, the DNA was subjected to amplification with the primer pairs. Specific primers or probes amplified only the target microbial species, genus, family or group.

Oligonucleotides primers found to amplify specifically the target species, genus, family or group were subsequently tested for their ubiquity by amplification (i.e. ubiquitous primers amplified efficiently most or all isolates of the target species or genus or family or group). Finally, the sensitivity of the primers or probes was determined by using 10-fold or 2-fold dilutions of purified genomic DNA from the targeted microorganism. For most assays, sensitivity levels in the

range of 1-100 copies were obtained. The specificity, ubiquity and sensitivity of the PCR assays using the selected amplification primer pairs were tested either directly from cultures of microbial species or from purified microbial genomic DNA.

Probes were tested in hybrid capture assays as described above. An oligonucleotide probe was considered specific only when it hybridized solely to DNA from the species or genus or family or group from which it was selected. Oligonucleotide probes found to be specific were subsequently tested for their ubiquity (i.e. ubiquitous probes detected efficiently most or all isolates of the target species or genus or family or group) by hybridization to microbial DNAs from different clinical isolates of the species or genus or family or group of interest including ATCC reference strains. Similarly, oligonucleotide primers and probes could be derived from antimicrobial agents resistance or toxin genes which are objects of the present invention.

Reference strains

The reference strains used to build proprietary *tuf*, *atpD* and *recA* sequence data subrepertories, as well as to test the amplification and hybridization assays were obtained from (i) the American Type Culture Collection (ATCC), (ii) the Laboratoire de santé publique du Québec (LSPQ), (iii) the Centers for Disease Control and Prevention (CDC), (iv) the National Culture Type Collection (NCTC) and (v) several other reference laboratories throughout the world. The identity of our reference strains was confirmed by phenotypic testing and reconfirmed by analysis of *tuf*, *atpD* and *recA* sequences (see Example 13).

Antimicrobial agents resistance genes

Antimicrobial resistance complicates treatment and often leads to therapeutic failures. Furthermore, overuse of antibiotics inevitably leads to the emergence of

microbial resistance. Our goal is to provide clinicians, in approximately one hour, the needed information to prescribe optimal treatments. Besides the rapid identification of negative clinical specimens with DNA-based tests for universal algal, archaeal, bacterial, fungal or parasitical detection and the identification of the presence of a specific pathogen in the positive specimens with species- and/or genus- and/or family- and/or group-specific DNA-based tests, clinicians also need timely information about the ability of the microbial pathogen to resist antibiotic treatments. We feel that the most efficient strategy to evaluate rapidly microbial resistance to antimicrobials is to detect directly from the clinical specimens the most common and clinically important antimicrobial agents resistance genes (i.e. DNA-based tests for the specific detection of antimicrobial agents resistance genes). Since the sequence from the most important and common antimicrobial agents resistance genes are available from public databases, our strategy is to use the sequence from a portion or from the entire resistance gene to design specific oligonucleotide primers or probes which will be used as a basis for the development of sensitive and rapid DNA-based tests. The list of each of the antimicrobial agents resistance genes selected on the basis of their clinical relevance (i.e. high incidence and importance) is given in Table 5; descriptions of the designed amplification primers and internal probes are given in Annexes XXXIV-XXXVII, XXXIX, XLV, and L-LI. Our approach is unique because the antimicrobial agents resistance genes detection and the microbial detection and identification can be performed simultaneously, or independently, or sequentially in multiplex or parallel or sequential assays under uniform PCR amplification conditions. These amplifications can also be done separately.

Toxin genes

Toxin identification is often very important to prescribe optimal treatments. Besides the rapid identification of negative clinical specimens with DNA-based tests for universal bacterial detection and the identification of the presence of a

specific pathogen in the positive specimens with species- and/or genus- and/or family- and/or group-specific DNA-based tests, clinicians sometimes need timely information about the ability of certain bacterial pathogens to produce toxins. Since the sequence from the most important and common bacterial toxin genes are available from public databases, our strategy is to use the sequence from a portion or from the entire toxin gene to design specific oligonucleotide primers or probes which will be used as a basis for the development of sensitive and rapid DNA-based tests. The list of each of the bacterial toxin genes selected on the basis of their clinical relevance (i.e. high incidence and importance) is given in Table 6; descriptions of the designed amplification primers and internal probes are given in Annexes XXII, XXXII and XXXIII. Our approach is unique because the toxin genes detection and the bacterial detection and identification can be performed simultaneously, or independently, or sequentially, in multiplex or parallel or sequential assays under uniform PCR amplification conditions. These amplifications can also be done separately.

Universal bacterial detection

In the routine microbiology laboratory, a high percentage of clinical specimens sent for bacterial identification are negative by culture. Testing clinical samples with universal amplification primers or universal probes to detect the presence of bacteria prior to specific identification and screening out the numerous negative specimens is thus useful as it reduces costs and may rapidly orient the clinical management of the patients. Several amplification primers and probes were therefore synthesized from highly conserved portions of bacterial sequences from the *tuf*, *atpD* and *recA* nucleic acids and/or sequences. The universal primers selection was based on a multiple sequence alignment constructed with sequences from our repertory.

All computer analysis of amino acid and nucleotide sequences were performed by using the GCG programs. Subsequently, optimal PCR primers for

the universal amplification of bacteria were selected with the help of the Oligo™ program. The selected primers are degenerated at several nucleotide positions and contain several inosines in order to allow the amplification of all clinically relevant bacterial species. Inosine is a nucleotide analog able to specifically bind to any of the four nucleotides A, C, G or T. Degenerated oligonucleotides consist of an oligonucleotide mix having two or more of the four nucleotides A, C, G or T at the site of mismatches. The inclusion of inosine and/or of base ambiguities in the amplification primers allow mismatch tolerance thereby permitting the amplification of a wider array of target nucleotide sequences (Dieffenbach and Dveksler, 1995 PCR Primer: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Plainview, NY).

The amplification conditions with the universal primers are very similar to those used for the species- and genus-specific amplification assays except that the annealing temperature is slightly lower. The original universal PCR assay described in our assigned WO98/20157 (SEQ ID NOs. 23-24 of the latter application) was specific and nearly ubiquitous for the detection of bacteria. The specificity for bacteria was verified by amplifying genomic DNA isolated from the 12 fungal species as well as genomic DNA from *Leishmania donovani*, *Saccharomyces cerevisiae* and human lymphocytes. None of the above eukaryotic DNA preparations could be amplified by the universal assay, thereby suggesting that this test is specific for bacteria. The ubiquity of the universal assay was verified by amplifying genomic DNAs from 116 reference strains which represent 95 of the most clinically relevant bacterial species. These species have been selected from the bacterial species listed in Table 4. We found that at least 104 of these strains could be amplified. However, the assay could be improved since bacterial species which could not be amplified with the original *tuf* nucleic acids and/or sequences-based assay included species belonging to the following genera: *Corynebacterium* (11 species) and *Stenotrophomonas* (1 species). Sequencing of the *tuf* genes from these bacterial species and others has been performed in the scope of the present invention in order to improve the universal assay. This

sequencing data has been used to select new universal primers which may be more ubiquitous and more sensitive. Also, we improved our primer and probes design strategy by taking into consideration the phylogeny observed in analysing our repertory of *tuf*, *atpD* and *recA* sequences. Data from each of the 3 main subrepertories (*tuf*, *atpD* and *recA*) was subjected to a basic phylogenic analysis using the Pileup command from version 10 of the GCG package (Genetics Computer Group, inc.). This analysis indicated the main branches or phyla reflecting the relationships between sequences. Instead of trying to design primers or probes able to hybridize to all phyla, we designed primers or probes able to hybridize to the main phyla while trying to use the largest phylum possible. This strategy should allow less degenerated primers hence improving sensitivity and by combining primers in a mutiplex assay, improve ubiquity. Universal primers SEQ ID NOs. 643-645 based on *tuf* sequences have been designed to amplify most pathogenic bacteria except *Actinomyceteae*, *Clostridiaceae* and the *Cytophaga*, *Flexibacter* and *Bacteroides* phylum (pathogenic bacteria of this phylum include mostly *Bacteroides*, *Porphyromonas* and *Prevotella* species). Primers to fill these gaps have been designed for *Actinomyceteae* (SEQ ID NOs. 646-648), *Clostridiaceae* (SEQ ID NOs. 796-797, 808-811), and the *Cytophaga*, *Flexibacter* and *Bacteroides* phylum (SEQ ID NOs. 649-651), also derived from *tuf* nucleic acids and/or sequences. These primers sets could be used alone or in conjuction to render the universal assay more ubiquitous.

Universal primers derived from *atpD* sequences include SEQ ID NOs. 562-565. Combination of these primers does not amplify human DNA but should amplify almost all pathogenic bacterial species except proteobacteria belonging to the epsilon subdivision (*Campylobacter* and *Helicobacter*), the bacteria from the *Cytophaga*, *Flexibacter* and *Bacteroides* group and some actinomycetes and corynebacteria. By analysing *atpD* sequences from the latter species, primers and probes to specifically fill these gaps could be designed and used in conjuction with primers SEQ ID NOs. 562-565, also derived from *atpD* nucleic acids and/or sequences.

In addition, universality of the assay could be expanded by mixing *atpD* sequences-derived primers with *tuf* sequences-derived primers. Ultimately, even *recA* sequences-derived primers could be added to fill some gaps in the universal assay.

It is important to note that the 95 bacterial species selected to test the ubiquity of the universal assay include all of the most clinically relevant bacterial species associated with a variety of human infections acquired in the community or in hospitals (nosocomial infections). The most clinically important bacterial and fungal pathogens are listed in Tables 1 and 2.

Amino acid sequences derived from *tuf*, *atpD* and *recA* nucleic acids and/or sequences

The amino acid sequences translated from the repertory of *tuf*, *atpD* and *recA* nucleic acids and/or sequences are also an object of the present invention. The amino acid sequence data will be particularly useful for homology modeling of three-dimensional (3D) structure of the elongation factor Tu, elongation factor G, elongation factor 1α, ATPase subunit beta and RecA recombinase. For all these proteins, at least one structure model has been published using X-ray diffraction data from crystals. Based on those structural informations it is possible to use computer sofware to build 3D model structures for any other protein having peptide sequence homologies with the known structure (Greer, 1991, Methods in Enzymology, **202**:239-252; Taylor, 1994, Trends Biotechnol., **12**(5):154-158; Sali, 1995, Curr. Opin. Biotechnol. **6**:437-451; Sanchez and Sali, 1997, Curr. Opin. Struct. Biol. **7**:206-214; Fischer and Eisenberg, 1999, Curr. Opin. Struct. Biol. **9**:208-211; Guex *et al.*, 1999, Trends Biochem. Sci. **24**: 364-367). Model structures of target proteins are used for the design or to predict the behavior of ligands and inhibitors such as antibiotics. Since EF-Tu and EF-G are already known as antibiotic targets (see above) and since the beta subunit of ATPase and RecA recombinase are essential to the survival of the microbial cells in natural

conditions of infection, all four proteins could be considered antibiotic targets. Sequence data, especially the new data generated by us could be very useful to assist the creation of new antibiotic molecules with desired spectrum of activity. In addition, model structures could be used to improve protein function for commercial purposes such as improving antibiotic production by microbial strains or increasing biomass.

The following detailed embodiments and appended drawings are provided as illustrative examples of his invention, with no intention to limit the scope thereof.

DESCRIPTION OF THE DRAWINGS

Figures 1 and 2 illustrate the principal subdivisions of the *tuf* and *atpD* sequences repertories, respectively. For the design of primers and probes, depending on the needs, one may want to use the complete data set illustrated on the top of the pyramid or use only a subset illustrated by the different branching points. Smaller subdivisions, representing groups, families, genus and species, could even be made to extend to the bottom of the pyramid. Because the *tuf* and *atpD* sequences are highly conserved and evolved with each species, the design of primers and probes does not need to include all the sequences within the database or its subdivisions. As illustrated in Annexes IV to XX, XXIII to XXXI, XXXVIII and XLII, depending on the use, sequences from a limited number of species can be carefully selected to represent: i) only the main phylogenetic branches from which the intended probes and primers need to be differentiating, and ii) only the species for which they need to be matching. However, for ubiquity purposes, and especially for primers and probes identifying large groups of species (genus, family, group or universal, or sequencing primers), the more data is included into the sequence analysis, the better the probes and primers will be suitable for each particular intended use. Similarly, for specificity purposes, a larger data set (or repertory) ensures optimal primers and probes design by reducing the chance of employing nonspecific oligonucleotides.

Figure 3 illustrates the approach used to design specific amplification primers from *fusA* as well as from the region between the end of *fusA* and the beginning of *tuf* in the streptomycin (*str*) operon (referred to as the *fusA-tuf* intergenic spacer in Table 7).

Figures 4 to 6 are illustrations to Example 42, whereas Figures 7 to 10 illustrate Example 43. Figures 11 and 12 illustrate Example 44.

FIGURE LEGENDS

Figure 3. Schematic organization of universal amplification primers (SEQ ID NOs. 1221-1229) in the *str* operon. Amplicon sizes are given in bases pairs. Drawing not to scale, as the *fusA-tuf* intergenic spacer size varies depending on the bacterial species. Indicated amplicon lengths are for *E. coli*.

Figure 4. Abridged multiple amino acid sequence alignment of the partial *tuf* gene products from selected species illustrated using the program Alscript. Residues highly conserved in bacteria are boxed in grey and gaps are represented with dots. Residues in reverse print are unique to the enterococcal *tufB* as well as to streptococcal and lactococcal *tuf* gene products. Numbering is based on *E. coli* EF-Tu and secondary structure elements of *E. coli* EF-Tu are represented by cylinders (α-helices) and arrows (β-strands).

Figure 5. Distance matrix tree of bacterial EF-Tu based on amino acid sequence homology. The tree was constructed by the neighbor-joining method. The tree was rooted using archeal and eukaryotic EF-1α genes as the outgroup. The scale bar represents 5% changes in amino acid sequence, as determined by taking the sum of all of the horizontal lines connecting two species.

Figure 6. Southern hybridization of *Bgl*II/*Xba*I digested genomic DNAs of some enterococci (except for *E. casseliflavus* and *E. gallinarum* whose genomic DNA was digested with *Bam*HI/*Pvu*II) using the *tufA* gene fragment of *E. faecium* as probes. The sizes of hybridizing fragments are shown in kilobases. Strains tested are listed in Table 16.

Figure 7. *Pantoea* and *Tatumella* species specific signature indel in *atpD* genes. The nucleotide positions given are for *E. coli atpD* sequence (GenBank accession no. V00267). Numbering starts from the first base of the initiation codon.

Figure 8: Trees based on sequence data from *tuf* (left side) and *atpD* (right side). The phylogenetic analysis was performed using the Neighbor-Joining method calculated using the Kimura two-parameter method. The value on each branch indicates the occurence (%) of the branching order in 750 bootstrapped trees.

Figure 9: Phylogenetic tree of members of the family *Enterobacteriaceae* based on *tuf* (a), *atpD* (b), and 16S rDNA (c) genes. Trees were generated by neighbor-joining method calculated using the Kimura two-parameter method. The value on each branch is the percentage of bootstrap replications supporting the branch. 750 bootstrap replications were calculated.

Figure 10: Plot of *tuf* distances versus 16S rDNA distances (a), *atpD* distances versus 16S rDNA distances (b), and *atpD* distances versus *tuf* distances (c). Symbols: ○, distances between pairs of strains belonging to the same species; , distances between *E. coli* strains and *Shigella* strains; □, distances between pairs belonging to the same genus; ■, distances between pairs belonging to different genera; △, distances between pairs belonging to different families.

EXAMPLES AND ANNEXES

For sake of clarity, here is a list of Examples and Annexes:

Example 1:	Sequencing of bacterial *atpD* (F-type and V-type) gene fragments.
Example 2:	Sequencing of eukaryotic *atpD* (F-type and V-type) gene fragments.
Example 3:	Sequencing of eukaryotic *tuf* (EF-1) gene fragments.

Example 4: Sequencing of eukaryotic *tuf* (organelle origin, M) gene fragments.

Example 5: Specific detection and identification of *Streptococcus agalactiae* using *tuf* sequences.

Example 6: Specific detection and identification of *Streptococcus agalactiae* using *atpD* sequences.

Example 7: Development of a PCR assay for detection and identification of staphylococci at genus and species levels.

Example 8: Differentiating between the two closely related yeast species *Candida albicans* and *Candida dubliniensis*.

Example 9: Specific detection and identification of *Entamoeba histolytica*.

Example 10: Sensitive detection and identification of *Chlamydia trachomatis*.

Example 11: Genus-specific detection and identification of enterococci.

Example 12: Detection and identification of the major bacterial platelets contaminants using *tuf* sequences with a multiplex PCR test.

Example 13: The resolving power of the *tuf* and *atpD* sequences databases is comparable to the biochemical methods for bacterial identification.

Example 14: Detection of group B streptococci from clinical specimens.

Example 15: Simultaneous detection and identification of *Streptococcus pyogenes* and its pyrogenic exotoxin A.

Example 16: Real-time detection and identification of Shiga toxin-producing bacteria.

Example 17: Development of a PCR assay for the detection and identification of staphylococci at genus and species levels and its associated *mecA* gene.

Example 18: Sequencing of *pbp1a, pbp2b* and *pbp2x* genes of *Streptoccoccus pneumoniae*.

Example 19: Sequencing of *hexA* genes of *Streptococcus* species.

Example 20: Development of a multiplex PCR assay for the detection of *Streptococcus pneumoniae* and its penicillin resistance genes.

Example 21: Sequencing of the vancomycin resistance *vanA*, *vanC1*, *vanC2* and *vanC3* genes.

Example 22: Development of a PCR assay for the detection and identification of enterococci at genus and species levels and its associated resistance genes *vanA* and *vanB*.

Example 23: Development of a multiplex PCR assay for detection and identification of vancomycin-resistant *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus gallinarum*, *Enterococcus casseliflavus*, and *Enterococcus flavescens*.

Example 24: Universal amplification involving the EF-G (*fusA*) subdivision of *tuf* sequences.

Example 25: DNA fragment isolation from *Staphylococcus saprophyticus* by arbitrarily primed PCR.

Example 26: Sequencing of prokaryotic *tuf* gene fragments.

Example 27: Sequencing of procaryotic *recA* gene fragments.

Example 28: Specific detection and identification of *Escherichia coli/Shigella* sp. using *tuf* sequences.

Example 29: Specific detection and identification of *Klebsiella pneumoniae* using *atpD* sequences.

Example 30: Specific detection and identification of *Acinetobacter baumanii* using *tuf* sequences.

Example 31: Specific detection and identification of *Neisseria gonorrhoeae* using *tuf* sequences.

Example 32: Sequencing of bacterial *gyrA* and *parC* gene fragments.

Example 33: Development of a PCR assay for the specific detection and identification of *Staphylococcus aureus* and its quinolone resistance genes *gyrA* and *parC*.

Example 34: Development of a PCR assay for the detection and identification of *Klebsiella pneumoniae* and its quinolone resistance genes *gyrA* and *parC*.

Example 35: Development of a PCR assay for the detection and identification of *Streptococcus pneumoniae* and its quinolone resistance genes *gyrA* and *parC*.

Example 36: Detection of extended-spectrum TEM-type β-lactamases in *Escherichia coli*.

Example 37: Detection of extended-spectrum SHV-type β-lactamases in *Klebsiella pneumoniae*.

Example 38: Development of a PCR assay for the detection and identification of *Neisseria gonorrhoeae* and its associated tetracycline resistance gene *tetM*.

Example 39: Development of a PCR assay for the detection and identification of *Shigella* sp. and their associated trimethoprim resistance gene *dhfr1a*.

Example 40: Development of a PCR assay for the detection and identification of *Acinetobacter baumanii* and its associated aminoglycoside resistance gene *aph(3')-VIa*.

Example 41: Specific detection and identification of *Bacteroides fragilis* using *atpD* (V-type) sequences.

Example 42: Evidence for horizontal gene transfer in the evolution of the elongation factor Tu in Enterococci.

Example 43: Elongation factor Tu (*tuf*) and the F-ATPase beta-subunit (*atpD*) as phylogenetic tools for species of the family *Enterobacteriaceae*.

Example 44: Testing new pairs of PCR primers selected from two species-specific genomic DNA fragments which are objects of US patent 6,001,564.

Example 45: Testing modified versions of PCR primers derived from the sequence of several primers which are objects of US patent 6,001,564.

The various Annexes show the strategies used for the selection of a variety of DNA amplification primers, nucleic acid hybridization probes and molecular beacon internal probes:

(i) Annex I shows the amplification primers used for nucleic acid amplification from *tuf* sequences.

(ii) Annex II shows the amplification primers used for nucleic acid amplification from *atpD* sequences.

(iii) Annex III shows the internal hybridization probes for detection of *tuf* sequences.

(iv) Annex IV illustrates the strategy used for the selection of the amplification primers specific for *atpD* sequences of the F-type.

(v) Annex V illustrates the strategy used for the selection of the amplification primers specific for *atpD* sequences of the V-type.

(vi) Annex VI illustrates the strategy used for the selection of the amplification primers specific for the *tuf* sequences of organelle lineage (M, the letter M is used to indicate that in most cases, the organelle is the mitochondria).

(vii) Annex VII illustrates the strategy used for the selection of the amplification primers specific for the *tuf* sequences of eukaryotes (EF-1).

(viii) Annex VIII illustrates the strategy for the selection of *Streptococcus agalactiae*-specific amplification primers from *tuf* sequences.

(ix) Annex IX illustrates the strategy for the selection of *Streptococcus agalactiae*-specific hybridization probes from *tuf* sequences.

(x) Annex X illustrates the strategy for the selection of *Streptococcus agalactiae*-specific amplification primers from *atp*D sequences.

(xi) Annex XI illustrates the strategy for the selection from *tuf* sequences of *Candida albicans/dubliniensis*-specific amplification primers, *Candida albicans*-specific hybridization probe and *Candida dubliniensis*-specific hybridization probe.

(xii) Annex XII illustrates the strategy for the selection of *Staphylococcus*-specific amplification primers from *tuf* sequences.

(xiii) Annex XIII illustrates the strategy for the selection of the *Staphylococcus*-specific hybridization probe from *tuf* sequences.

(xiv) Annex XIV illustrates the strategy for the selection of *Staphylococcus saprophyticus*-specific and *Staphylococcus haemolyticus*-specific hybridization probes from *tuf* sequences.

(xv) Annex XV illustrates the strategy for the selection of *Staphylococcus aureus*-specific and *Staphylococcus epidermidis*-specific hybridization probes from *tuf* sequences.

(xvi) Annex XVI illustrates the strategy for the selection of the *Staphylococcus hominis*-specific hybridization probe from *tuf* sequences.

(xvii) Annex XVII illustrates the strategy for the selection of the *Enterococcus*-specific amplification primers from *tuf* sequences.

(xviii) Annex XVIII illustrates the strategy for the selection of the *Enterococcus faecalis*-specific hybridization probe, of the *Enterococcus faecium*-specific hybridization probe and of the *Enterococcus casseliflavus-flavescens-gallinarum* group-specific hybridization probe from *tuf* sequences.

(xix) Annex XIX illustrates the strategy for the selection of primers from *tuf* sequences for the identification of platelets contaminants.

(xx) Annex XX illustrates the strategy for the selection of the universal amplification primers from *atpD* sequences.

(xxi) Annex XXI shows the amplification primers used for nucleic acid amplification from *rec*A sequences.

(xxii) Annex XXII shows the specific and ubiquitous primers for nucleic acid amplification from *speA* sequences.

(xxiii) Annex XXIII illustrates the first strategy for the selection of *Streptococcus pyogenes*-specific amplification primers from *speA* sequences.

(xxiv) Annex XXIV illustrates the second strategy for the selection of *Streptococcus pyogenes*-specific amplification primers from *speA* sequences.

(xxv) Annex XXV illustrates the strategy for the selection of *Streptococcus pyogenes*-specific amplification primers from *tuf* sequences.

(xxvi) Annex XXVI illustrates the strategy for the selection of stx_1-specific amplification primers and hybridization probe.

(xxvii) Annex XXVII illustrates the strategy for the selection of stx_2-specific amplification primers and hybridization probe.

(xxviii) Annex XXVIII illustrates the strategy for the selection of *vanA*-specific amplification primers from *van* sequences.

(xxix) Annex XXIX illustrates the strategy for the selection of *vanB*-specific amplification primers from *van* sequences.

(xxx) Annex XXX illustrates the strategy for the selection of *vanC*-specific amplification primers from *vanC* sequences.

(xxxi) Annex XXXI illustrates the strategy for the selection of *Streptococcus pneumoniae*-specific amplification primers and hybridization probes from *pbp1a* sequences.

(xxxii) Annex XXXII shows the specific and ubiquitous primers for nucleic acid amplification from toxin gene sequences.

(xxxiii) Annex XXXIII shows the molecular beacon internal hybridization probes for specific detection of toxin sequences.

(xxxiv) Annex XXXIV shows the specific and ubiquitous primers for nucleic acid amplification from *van* sequences.

(xxxv) Annex XXXV shows the internal hybridization probes for specific detection of *van* sequences.

(xxxvi) Annex XXXVI shows the specific and ubiquitous primers for nucleic acid amplification from *pbp* sequences.

(xxxvii) Annex XXXVII shows the internal hybridization probes for specific detection of *pbp* sequences.

(xxxviii) Annex XXXVIII illustrates the strategy for the selection of *vanAB*-specific amplification primers and *vanA*- and *vanB*- specific hybridization probes from *van* sequences.

(xxxix) Annex XXXIX shows the internal hybridization probe for specific detection of *mecA*.

(xl) Annex XL shows the specific and ubiquitous primers for nucleic acid amplification from *hexA* sequences.

(xli) Annex XLI shows the internal hybridization probe for specific detection of *hexA*.

(xlii) Annex XLII illustrates the strategy for the selection of *Streptococcus pneumoniae* species-specific amplification primers and hybridization probe from *hexA* sequences.

(xliii) Annex XLIII shows the specific and ubiquitous primers for nucleic acid amplification from *pcp* sequences.

(xliv) Annex XLIV shows specific and ubiquitous primers for nucleic acid amplification of *S. saprophyticus* sequences of unknown coding potential.

(xlv) Annex XLV shows the molecular beacon internal hybridization probes for specific detection of antimicrobial agents resistance gene sequences.

(xlvi) Annex XLVI shows the molecular beacon internal hybridization probe for specific detection of *S. aureus* gene sequences of unknown coding potential.

(xlvii) Annex XLVII shows the molecular beacon hybridization internal probe for specific detection of *tuf* sequences.

(xlviii) Annex XLVIII shows the molecular beacon internal hybridization probes for specific detection of *ddl* and *mtl* sequences.

(xlix) Annex XLIX shows the internal hybridization probe for specific detection of *S. aureus* sequences of unknown coding potential.

(l) Annex L shows the amplification primers used for nucleic acid amplification from antimicrobial agents resistance genes sequences.

(li) Annex LI shows the internal hybridization probes for specific detection of antimicrobial agents resistance genes sequences.

(lii) Annex LII shows the molecular beacon internal hybridization probes for specific detection of *atpD* sequences.

(liii) Annex LIII shows the internal hybridization probes for specific detection of *atpD* sequences.

(liv) Annex LIVI shows the internal hybridization probes for specific detection of *ddl* and *mtl* sequences.

As shown in these Annexes, the selected amplification primers may contain inosines and/or base ambiguities. Inosine is a nucleotide analog able to specifically bind to any of the four nucleotides A, C, G or T. Alternatively, degenerated oligonucleotides which consist of an oligonucleotide mix having two or more of the four nucleotides A, C, G or T at the site of mismatches were used. The inclusion of inosine and/or of degeneracies in the amplification primers allows mismatch tolerance thereby permitting the amplification of a wider array of target nucleotide sequences (Dieffenbach and Dveksler, 1995 PCR Primer: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Plainview, New York).

EXAMPLES

EXAMPLE 1:

Sequencing of bacterial *atpD* (F-type and V-type) gene fragments. As shown in Annex IV, the comparison of publicly available *atpD* (F-type) sequences from a variety of bacterial species revealed conserved regions allowing the design of PCR primers able to amplify *atpD* sequences (F-type) from a wide range of bacterial species. Using primers pairs SEQ ID NOs. 566 and 567, 566 and 814, 568 and 567, 570 and 567, 572 and 567, 569 and 567, 571 and 567, 700 and 567, it was possible to amplify and sequence *atpD* sequences SEQ ID NOs. 242-270, 272-398, 673-

674, 737-767, 866-867, 942-955, 1245-1254, 1256-1265, 1527, 1576, 1577, 1600-1604, 1640-1646, 1649, 1652, 1655, 1657, 1659-1660, 1671, 1844-1845, and 1849-1865.

Similarly, Annex V shows the strategy to design the PCR primers able to amplify *atpD* sequences of the V-type from a wide range of archaeal and bacterial species. Using primers SEQ ID NOs. 681-683, it was possible to amplify and sequence *atpD* sequences SEQ ID NOs. 827-832, 929-931, 958 and 966. As the gene was difficult to amplify for several species, additional amplification primers were designed inside the original amplicon (SEQ ID NOs. 1203-1207) in order to obtain sequence information for these species. Other primers (SEQ ID NO. 1212, 1213, 2282-2285) were also designed to amplify regions of the *atpD* gene (V-type) in archaebacteria.

EXAMPLE 2:

Sequencing of eukaryotic *atpD* (F-type and V-type) gene fragments. The comparison of publicly available *atpD* (F-type) sequences from a variety of fungal and parasitical species revealed conserved regions allowing the design of PCR primers able to amplify *atpD* sequences from a wide range of fungal and parasitical species. Using primers pairs SEQ ID NOs. 568 and 573, 574 and 573, 574 and 708, and 566 and 567, it was possible to amplify and sequence *atpD* sequences SEQ ID NOs. 458-497, 530-538, 663, 667, 676, 678-680, 768-778, 856-862, 889-896, 941, 1638-1639, 1647, 1650-1651, 1653-1654, 1656, 1658, 1684, 1846-1848, and 2189-2192.

In the same manner, the primers described in Annex V (SEQ ID NOs. 681-683) could amplify the *atpD* (V-type) gene from various fungal and parasitical species. This strategy allowed to obtain SEQ ID NOs. 834-839, 956-957, and 959-965.

EXAMPLE 3:

Sequencing of eukaryotic *tuf* (EF-1) gene fragments. As shown in Annex VII, the comparison of publicly available *tuf* (EF-1) sequences from a variety of fungal and parasitical species revealed conserved regions allowing the design of PCR primers able to amplify *tuf* sequences from a wide range of fungal and parasitical species. Using primers pairs SEQ ID NOs. 558 and 559, 813 and 559, 558 and 815, 560 and 559, 653 and 559, 558 and 655, and 654 and 559, 1999 and 2000, 2001 and 2003, 2002 and 2003, it was possible to amplify and sequence *tuf* sequences SEQ ID NOs. 399-457, 509-529, 622-624, 677, 779-790, 840-842, 865, 897-903, 1266-1287, 1561-1571 and 1685.

EXAMPLE 4:

Sequencing of eukaryotic *tuf* (organelle origin, M) gene fragments. As shown in Annex VI, the comparison of publicly available *tuf* (organelle origin, M) sequences from a variety of fungal and parasitical organelles revealed conserved regions allowing the design of PCR primers able to amplify *tuf* sequences of several organelles belonging to a wide range fungal and parasitical species. Using primers pairs SEQ ID NOs. 664 and 652, 664 and 561, 911 and 914, 912 and 914, 913 and 915, 916 and 561, 664 and 917, it was possible to amplify and sequence *tuf* sequences SEQ ID NOs. 498-508, 791-792, 843-855, 904-910, 1664, 1666-1667, 1669-1670, 1673-1683, 1686-1689, 1874-1876, 1879, 1956-1960, and 2193-2199.

EXAMPLE 5:

Specific detection and identification of *Streptococcus agalactiae* using *tuf* sequences. As shown in Annex VIII, the comparison of *tuf* sequences from a variety of bacterial species allowed the selection of PCR primers specific for *S. agalactiae*. The strategy used to design the PCR primers was based on the analysis

of a multiple sequence alignment of various *tuf* sequences. The multiple sequence alignment includes the *tuf* sequences of four bacterial strains from the target species as well as *tuf* sequences from other species and bacterial genera, especially representatives of closely related species. A careful analysis of this alignment allowed the selection of oligonucleotide sequences which are conserved within the target species but which discriminate sequences from other species and genera, especially from the closely related species, thereby permitting the species-specific, ubiquitous and sensitive detection and identification of the target bacterial species.

The chosen primer pair, oligos SEQ ID NO. 549 and SEQ ID NO. 550, gives an amplification product of 252 bp. Standard PCR was carried out using 0.4 μM of each primer, 2.5 mM $MgCl_2$, BSA 0.05 mM, 1X Taq Buffer (Promega), dNTP 0.2 mM (Pharmacia), 0,5 U *Taq* DNA polymerase (Promega) coupled with TaqStart™ antibody (Clontech Laboratories Inc., Palo Alto), 1 μl of genomic DNA sample in a final volume of 20 μl using a PTC-200 thermocycler (MJ Research Inc.). The optimal cycling conditions for maximum sensitivity and specificity were 3 minutes at 95 °C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 62 °C, followed by terminal extension at 72 °C for 2 minutes. Detection of the PCR products was made by electrophoresis in agarose gels (2 %) containing 0.25 μg/ml of ethidium bromide.

Specificity of the assay was tested by adding into the PCR reactions, 0.1 ng of genomic DNA from each of the bacterial species listed in Table 8. Efficient amplification was observed only for the 5 *S. agalactiae* strains listed. Of the other bacterial species, including 32 species representative of the vaginal flora and 27 other streptococcal species, only *S. acidominimus* yielded amplification. The signal with 0.1 ng of *S. acidominimus* genomic DNA was weak and the detection limit for this species was 10 pg (corresponding to more than 4000 genome copies) while the detection limit for *S. agalactiae* was 2.5 fg (corresponding to one genome copy) of genomic DNA.

To increase the specificity of the assay, internal probes were designed for FRET (Fluorescence Resonance Energy Transfer) detection using the LightCycler™ (Idaho Technology). As illustrated in Annex IX, a multiple sequence alignment of streptococcal *tuf* sequence fragments corresponding to the 252 bp region amplified by primers SEQ ID NO. 549 and SEQ ID NO. 550, was used for the design of internal probes TSagHF436 (SEQ ID NO. 582) and TSagHF465 (SEQ ID NO. 583). The region of the amplicon selected for internal probes contained sequences unique and specific to *S. agalactiae*. SEQ ID NO. 583, the more specific probe, is labelled with fluorescein in 3', while SEQ ID NO. 582, the less discriminant probe, is labelled with CY5 in 5' and blocked in 3' with a phosphate group. However, since the FRET signal is only emitted if both probes are adjacently hybridized on the same target amplicon, detection is highly specific.

Real-time detection of PCR products using the LightCycler™ was carried out using 0.4 μM of each primer (SEQ ID NO. 549-550), 0.2 μM of each probe (SEQ ID NO. 582-583), 2.5 mM $MgCl_2$, BSA 450 μg/ml, 1X PC2 Buffer (AB Peptides, St-Louis, MO), dNTP 0.2 mM (Pharmacia), 0.5 U KlenTaq1™ DNA polymerase (AB Peptides) coupled with TaqStart™ antibody (Clontech Laboratories Inc., Palo Alto), 0.7 μl of genomic DNA sample in a final volume of 7 μl using a LightCycler thermocycler (Idaho Technology). The optimal cycling conditions for maximum sensitivity and specificity were 3 minutes at 94 °C for initial denaturation, then forty cycles of three steps consisting of 0 second (this setting meaning the LightCycler will reach the target temperature and stay at it for its minimal amount of time) at 94 °C, 10 seconds at 64 °C, 20 seconds at 72 °C. Amplification was monitored during each annealing steps using the fluorescence ratio. The streptococcal species having close sequence homologies with the *tuf* sequence of *S. agalactiae* (*S. acidominimus, S. anginosus, S. bovis, S. dysgalactiae, S. equi, S. ferus, S. gordonii, S. intermedius, S. parasanguis, S. parauberis, S. salivarius, S. sanguis, S. suis*) as well as *S. agalactiae* were tested in the

LightCycler with 0.07 ng of genomic DNA per reaction. Only *S. agalactiae* yielded an amplification signal, hence demonstrating that the assay is species-specific. With the LightCycler™ assay using the internal FRET probes, the detection limit for *S. agalactiae* was 1-2 genome copies of genomic DNA.

EXAMPLE 6:

Specific detection and identification of *Streptococcus agalactiae* using *atpD* sequences. As shown in Annex X, the comparison of *atpD* sequences from a variety of bacterial species allowed the selection of PCR primers specific for *S. agalactiae*. The primer design strategy is similar to the strategy described in the preceding Example except that *atpD* sequences were used in the alignment.

Four primers were selected, ASag42 (SEQ ID NO. 627), ASag52 (SEQ ID NO. 628), ASag206 (SEQ ID NO. 625) and ASag371 (SEQ ID NO. 626). The following combinations of these four primers give four amplicons; SEQ ID NO. 627 + SEQ ID NO. 625 = 190 bp, SEQ ID NO. 628 + SEQ ID NO. 625 = 180 bp, SEQ ID NO. 627 + SEQ ID NO. 626 = 355 bp, and SEQ ID NO. 628 + SEQ ID NO. 626 = 345 bp. 。

Standard PCR was carried out on PTC-200 thermocyclers (MJ Research Inc) using 0.4 μM of each primers pair, 2.5 mM $MgCl_2$, BSA 0.05 mM, 1X *taq* Buffer (Promega), dNTP 0.2 mM (Pharmacia), 0.5 U *Taq* DNA polymerase (Promega) coupled with TaqStart™ antibody (Clontech Laboratories Inc., Palo Alto), 1 μl of genomic DNA sample in a final volume of 20 μL. The optimal cycling conditions for maximum sensitivity and specificity were adjusted for each primer pair. Three minutes at 95 °C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at the optimal annealing temperature specified below were followed by terminal extension at 72 °C for 2 minutes. Detection of the PCR products was made by electrophoresis in agarose gels (2 %) containing

0.25 μg/ml of ethidium bromide. Since *atpD* sequences are relatively more specific than *tuf* sequences, only the most closely related species namely, the steptococcal species listed in Table 9, were tested.

All four primer pairs only amplified the six *S. agalactiae* strains. With an annealing temperature of 63 °C, the primer pair SEQ ID NO. 627 + SEQ ID NO. 625 had a sensitivity of 1-5 fg (equivalent to 1-2 genome copies). At 55 °C, the primer pair SEQ ID NO. 628 + SEQ ID NO. 625 had a sensitivity of 2.5 fg (equivalent to 1 genome copy). At 60 °C, the primer pair SEQ ID NO. 627 + SEQ ID NO. 626 had a sensitivity of 10 fg (equivalent to 4 genome copies). At 58 °C, the primer pair SEQ ID NO. 628 + SEQ ID NO. 626 had a sensitivity of 2.5-5 fg (equivalent to 1-2 genome copies). This proves that all four primer pairs can detect *S. agalactiae* with high specificity and sensitivity. Together with Example 5, this example demonstrates that both *tuf* and *atpD* sequences are suitable and flexible targets for the identification of microorganisms at the species level. The fact that 4 different primer pairs based on *atpD* sequences led to efficient and specific amplification of *S. agalactiae* demonstrates that the challenge is to find target genes suitable for diagnostic purposes, rather than finding primer pairs from these target sequences.

EXAMPLE 7:

Development of a PCR assay for detection and identification of staphylococci at genus and species levels.

Materials and Methods

Bacterial strains. The specificity of the PCR assay was verified by using a panel of ATCC (America Type Culture Collection) and DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; German Collection of

Microorganisms and Cell Cultures) reference strains consisting of 33 gram-negative and 47 gram-positive bacterial species (Table 12). In addition, 295 clinical isolates representing 11 different species of staphylococci from the microbiology laboratory of the Centre Hospitalier Universitaire de Québec, Pavillon Centre Hospitalier de l'Université Laval (CHUL) (Ste-Foy, Québec, Canada) were also tested to further validate the *Staphylococcus*-specific PCR assay. These strains were all identified by using (i) conventional methods or (ii) the automated MicroScan Autoscan-4 system equipped with the Positive BP Combo Panel Type 6 (Dade Diagnostics, Mississauga, Ontario, Canada). Bacterial strains from frozen stocks kept at –80 °C in brain heart infusion (BHI) broth containing 10% glycerol were cultured on sheep blood agar or in BHI broth (Quelab Laboratories Inc, Montréal, Québec, Canada).

PCR primers and internal probes. Based on multiple sequence alignments, regions of the *tuf* gene unique to staphylococci were identified. *Staphylococcus*-specific PCR primers TStaG422 (SEQ ID NO. 553) and TStaG765 (SEQ ID NO. 575) were derived from these regions (Annex XII). These PCR primers are displaced by two nucleotide positions compared to original *Staphylococcus*-specific PCR primers described in our patent publication WO98/20157 (SEQ ID NOs. 17 and 20 in the said patent publication). These modifications were done to ensure specificity and ubiquity of the primer pair, in the light of new *tuf* sequence data revealed in the present patent application for several additional staphylococcal species and strains.

Similarly, sequence alignment analysis were performed to design genus and species-specific internal probes (see Annexes XIII to XVI). Two internal probes specific for *Staphylococcus* (SEQ ID NOs. 605-606), five specific for *S. aureus* (SEQ ID NOs. 584-588), five specific for *S. epidermidis* (SEQ ID NO. 589-593), two specific for *S. haemolyticus* (SEQ ID NOs. 594-595), three specific for *S. hominis* (SEQ ID NOs. 596-598), four specific for *S. saprophyticus* (SEQ ID NOs. 599-601 and 695), and two specific for coagulase-negative *Staphylococcus* species including

S. epidermidis, *S. hominis*, *S. saprophyticus*, *S. auricularis*, *S. capitis*, *S. haemolyticus*, *S. lugdunensis*, *S. simulans*, *S. cohnii* and *S. warneri* (SEQ ID NOs. 1175-1176) were designed. The range of mismatches between the *Staphylococcus*-specific 371-bp amplicon and each of the 20-mer species-specific internal probes was from 1 to 5, in the middle of the probe when possible. No mismatches were present in the two *Staphylococcus*-specific probes for the 11 species analyzed: *S. aureus, S. auricularis, S. capitis, S. cohnii, S. epidermidis, S. haemolyticus, S. hominis, S. lugdunensis, S. saprophyticus, S. simulans* and *S. warneri.* In order to verify the intra-specific sequence conservation of the nucleotide sequence, sequences were obtained for the 371-bp amplicon from five unrelated ATCC and clinical strains for each of the species *S. aureus, S. epidermidis, S. haemolyticus, S. hominis* and *S. saprophyticus.* The Oligo™ (version 5.0) primer analysis software (National Biosciences, Plymouth, Minn.) was used to confirm the absence of self-complementary regions within and between the primers or probes. When required, the primers contained inosines or degenerated nucleotides at one or more variable positions. Oligonucleotide primers and probes were synthesized on a model 394 DNA synthesizer (Applied Biosystems, Mississauga, Ontario, Canada). Detection of the hybridization was performed with the DIG-labeled dUTP incorporated during amplification with the *Staphylococcus*-specific PCR assay, and the hybridization signal was detected with a luminometer (Dynex Technologies) as described above in the section on luminescent detection of amplification products. Annexes XIII to XVI illustrate the strategy for the selection of several internal probes.

PCR amplification. For all bacterial species, amplification was performed from purified genomic DNA or from a bacterial suspension whose turbidity was adjusted to that of a 0.5 McFarland standard, which corresponds to approximately 1.5 x 10^8 bacteria per ml. One nanogram of genomic DNA or 1 µl of the standardized bacterial suspension was transferred directly to a 19 µl PCR mixture. Each PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM

$MgCl_2$, 0.2 µM (each) of the two *Staphylococcus* genus-specific primers (SEQ ID NOs. 553 and 575), 200 µM (each) of the four deoxynucleoside triphosphates (Pharmacia Biotech), 3.3 µg/µl bovine serum albumin (BSA) (Sigma-Aldrich Canada Ltd, Oakville, Ontario, Canada), and 0.5 U *Taq* polymerase (Promega) coupled with *Taq*Start™ Antibody (Clontech). The PCR amplification was performed as follows: 3 min. at 94 °C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 55 °C, plus a terminal extension at 72 °C for 2 minutes. Detection of the PCR products was made by electrophoresis in agarose gels (2 %) containing 0.25 µg/ml of ethidium bromide. Visualization of the PCR products was made under UV at 254 nm.

For determination of the sensitivities of the PCR assays, two-fold dilutions of purified genomic DNA were used to determine the minimal number of genome copies which can be detected.

Results

Amplifications with the *Staphylococcus* genus-specific PCR assay. The specificity of the assay was assessed by performing 30-cycle and 40-cycle PCR amplifications with the panel of gram-positive (47 species from 8 genera) and gram-negative (33 species from 22 genera) bacterial species listed in Table 12. The PCR assay was able to detect efficiently 27 of 27 staphylococcal species tested in both 30-cycle and 40-cycle regimens. For 30-cycle PCR, all bacterial species tested other than staphylococci were negative. For 40-cycle PCR, *Enterococcus faecalis* and *Macrococcus caseolyticus* were slightly positive for the *Staphylococcus*-specific PCR assay. The other species tested remained negative. Ubiquity tests performed on a collection of 295 clinical isolates provided by the microbiology laboratory of the Centre Hospitalier Universitaire de Québec, Pavillon Centre Hospitalier de l'Université Laval (CHUL), including *Staphylococcus aureus* (n=34), *S. auricularis* (n=2), *S. capitis* (n=19), *S. cohnii* (n=5), *S. epidermidis* (n=18), *S. haemolyticus*

(n=21), *S. hominis* (n=73), *S. lugdunensis* (n=17), *S. saprophyticus* (n=6), *S. simulans* (n=3), *S. warneri* (n=32) and *Staphylococcus* sp. (n=65), showed a uniform amplification signal with the 30-cycle PCR assays and a perfect relation between the genotype and classical identification schemes.

The sensitivity of the *Staphylococcus*-specific assay with 30-cycle and 40-cycle PCR protocols was determined by using purified genomic DNA from the 11 staphylococcal species previously mentioned. For PCR with 30 cycles, a detection limit of 50 copies of genomic DNA was consistently obtained. In order to enhance the sensitivity of the assay, the number of cycles was increased. For 40-cycle PCR assays, the detection limit was lowered to a range of 5-10 genome copies, depending on the staphylococcal species tested.

Hybridization between the *Staphylococcus*-specific 371-bp amplicon and species-specific or genus-specific internal probes. Inter-species polymorphism was sufficient to generate species-specific internal probes for each of the principal species involved in human diseases (*S. aureus, S. epidermidis, S. haemolyticus, S. hominis* and *S. saprophyticus*). In order to verify the intra-species sequence conservation of the nucleotide sequence, sequence comparisons were performed on the 371-bp amplicon from five unrelated ATCC and clinical strains for each of the 5 principal staphylococcal species: *S. aureus, S. epidermidis, S. haemolyticus, S. hominis* and *S. saprophyticus*. Results showed a high level of conservation of nucleotide sequence between different unrelated strains from the same species. This sequence information allowed the development of staphylococcal species identification assays using species-specific internal probes hybridizing to the 371-bp amplicon. These assays are specific and ubiquitous for those five staphylococcal species. In addition to the species-specific internal probes, the genus-specific internals probes were able to recognize all or most *Staphylococcus* species tested.

EXAMPLE 8:

Differentiating between the two closely related yeast species *Candida albicans* and *Candida dubliniensis*. It is often useful for the clinician to be able to differentiate between two very closely related species of microorganisms. *Candida albicans* is the most important cause of invasive human mycose. In recent years, a very closely related species, *Candida dubliniensis*, was isolated in immunosuppressed patients. These two species are difficult to distinguish by classic biochemical methods. This example demonstrates the use of *tuf* sequences to differentiate *Candida albicans* and *Candida dubliniensis*. PCR primers SEQ ID NOs. 11-12, from previous patent publication WO98/20157, were selected for their ability to specifically amplify a *tuf* (elongation factor 1 alpha type) fragment from both species (see Annex XI for primer positions). Within this *tuf* fragment, a region differentiating *C. albicans* and *C. dubliniensis* by two nucleotides was selected and used to design two internal probes (see Annex XI for probe design, SEQ ID NOs. 577 and 578) specific for each species. Amplification of genomic DNA from *C. albicans* and *C. dubliniensis* was carried out using DIG-11-dUTP as described above in the section on chemiluminescent detection of amplification products. Internal probes SEQ ID NOs. 577 and 578 were immobilized on the bottom of individual microtiter plates and hybridization was carried out as described above in the above section on chemiluminescent detection of amplification products. Luminometer data showed that the amplicon from *C. albicans* hybridized only to probe SEQ ID NO. 577 while the amplicon from *C. dubliniensis* hybridized only to probe SEQ ID NO. 578, thereby demonstrating that each probe was species-specific.

EXAMPLE 9:

Specific identification of *Entamoeba histolytica*. Upon analysis of *tuf* (elongation factor 1 alpha) sequence data, it was possible to find four regions where

Entamoeba histolytica sequences remained conserved while other parasitical and eukaryotic species have diverged. Primers TEntG38 (SEQ ID NO. 703), TEntG442 (SEQ ID NO. 704), TEntG534 (SEQ ID NO. 705), and TEntG768 (SEQ ID NO. 706) were designed so that SEQ ID NO. 703 could be paired with the three other primers. On PTC-200 thermocyclers (MJ Research), the cycling conditions for initial sensitivity and specificity testing were 3 min. at 94 °C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 55 °C, followed by terminal extension at 72 °C for 2 minutes. Detection of the PCR products was made by electrophoresis in agarose gels (2 %) containing 0.25 μg/ml of ethidium bromide. The three primer pairs could detect the equivalent of less than 200 *E. histolytica* genome copies. Specificity was tested using 0.5 ng of purified genomic DNA from a panel of microorganisms including *Babesia bovis*, *Babesia microtti*, *Candida albicans*, *Crithidia fasciculata*, *Leishmania major*, *Leishmania hertigi* and *Neospora caninum*. Only *E. histolytica* DNA could be amplified, thereby suggesting that the assay was species-specific.

EXAMPLE 10:

<u>Sensitive identification of *Chlamydia trachomatis*</u>. Upon analysis of *tuf* sequence data, it was possible to find two regions where *Chlamydia trachomatis* sequences remained conserved while other species have diverged. Primers Ctr82 (SEQ ID NO. 554) and Ctr249 (SEQ ID NO. 555) were designed. With the PTC-200 thermocyclers (MJ Research), the optimal cycling conditions for maximum sensitivity and specificity were determined to be 3 min. at 94 °C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 60 °C, followed by terminal extension at 72 °C for 2 minutes. Detection of the PCR products was made by electrophoresis in agarose gels (2 %) containing 0.25 μg/ml of ethidium bromide. The assay could detect the equivalent of 8 *C. trachomatis* genome copies. Specificity was tested with 0.1 ng of purified genomic DNA from a panel of microorganisms including 22 species commonly encountered

in the vaginal flora (*Bacillus subtilis, Bacteroides fragilis, Candida albicans, Clostridium difficile, Corynebacterium cervicis, Corynebacterium urealyticum, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Fusobacterium nucleatum, Gardnerella vaginalis, Haemophilus influenzae, Klebsiella oxytoca, Lactobacillus acidophilus, Peptococcus niger, Peptostreptococcus prevotii, Porphyromonas asaccharolytica, Prevotella melaninogenica, Propionibacterium acnes, Staphylococcus aureus, Streptococcus acidominimus,* and *Streptococcus agalactiae*). Only *C. trachomatis* DNA could be amplified, thereby suggesting that the assay was species-specific.

EXAMPLE 11:

Genus-specific detection and identification of enterococci. Upon analysis of *tuf* sequence data and comparison with the repertory of *tuf* sequences, it was possible to find two regions where *Enterococcus* sequences remained conserved while other genera have diverged (Annex XVII). Primer pair Encg313dF and Encg599c (SEQ ID NOs. 1137 and 1136) was tested for its specificity by using purified genomic DNA from a panel of bacteria listed in Table 10. Using the PTC-200 thermocycler (MJ Research), the optimal cycling conditions for maximum sensitivity and specificity were determined to be 3 min. at 94 °C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 55 °C, followed by terminal extension at 72 °C for 2 minutes. Detection of the PCR products was made by electrophoresis in agarose gels (2 %) containing 0.25 μg/ml of ethidium bromide. Visualization of the PCR products was made under UV at 254 nm. The 18 enterococcal species listed in Table 10 were all amplified efficiently. The only other species amplified were *Abiotrophia adiacens, Gemella haemolysans* and *Gemella morbillorum*, three gram-positive species. Sensitivity tested with several strains of *E. casseliflavus, E. faecium, E. faecalis, E. flavescens* and *E. gallinarum* and with one strain of each other *Enterococcus* species listed in Table 10 ranged from 1 to 10 copies of genomic DNA. The sequence variation

within the 308-bp amplicon was sufficient so that internal probes could be used to speciate the amplicon and differenciate enterococci from *Abiotrophia adiacens, Gemella haemolysans* and *Gemella morbillorum*, thereby allowing to achieve excellent specificity. Species-specific internal probes were generated for each of the clinically important species, *E. faecalis* (SEQ ID NO. 1174), *E. faecium* (SEQ ID NO. 602), and the group including *E. casseliflavus, E. flavescens* and *E. gallinarum* (SEQ ID NO. 1122) (Annex XVIII). The species-specific internal probes were able to differentiate their respective *Enterococcus* species from all other *Enterococcus* species. These assays are sensitive, specific and ubiquitous for those five *Enterococcus* species.

EXAMPLE 12:

Identification of the major bacterial platelets contaminants using *tuf* sequences with a multiplex PCR test. Blood platelets preparations need to be monitored for bacterial contaminations. The *tuf* sequences of 17 important bacterial contaminants of platelets were aligned. As shown in Annex XIX, analysis of these sequences allowed the design of PCR primers. Since in the case of contamination of platelet concentrates, detecting all species (not just the more frequently encountered ones) is desirable, perfect specificity of primers was not an issue in the design. However, sensitivity is important. That is why, to avoid having to put too much degeneracy, only the most frequent contaminants were included in primer design, knowing that the selected primers would anyway be able to amplify more species than the 17 used in the design because they target highly conserved regions of *tuf* sequences. Oligonucleotide sequences which are conserved in these 17 major bacterial contaminants of platelet concentrates were chosen (oligos Tplaq 769 and Tplaq 991, respectively SEQ ID NOs. 636 and 637) thereby permitting the detection of these bacterial species. However, sensitivity was slightly deficient with staphylococci. To ensure maximal sensitivity in the detection of all the more frequent bacterial contaminants, a multiplex assay also including oligonucleotide

primers targetting the *Staphylococcus* genera (oligos Stag 422, SEQ ID NO. 553; and Stag 765, SEQ ID NO. 575) was developed. The bacterial species detected with the assay are listed in Table 14.

The primer pairs, oligos SEQ ID NO. 636 and SEQ ID NO. 637 that give an amplification product of 245 pb, and oligos SEQ ID NO. 553 and SEQ ID NO. 575 that give an amplification product of 368 pb, were used simultaneously in the multiplex PCR assay. Detection of these PCR products was made on the LightCycler thermocycler (Idaho Technology) using SYBR® Green I (Molecular Probe Inc.). SYBR® Green I is a fluorescent dye that binds specifically to double-stranded DNA.

Fluorogenic detection of PCR products with the LightCycler was carried out using 1.0 μM of both Tplaq primers (SEQ ID NOs. 636-637) and 0.4 μM of both TStaG primers (SEQ ID NOs. 553 and 575), 2.5 mM $MgCl_2$, BSA 7.5 μM , dNTP 0.2 mM (Pharmacia), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.5 U *Taq* DNA polymerase (Boerhinger Mannheim) coupled with TaqStart™ antibody (Clontech), and 0.07 ng of genomic DNA sample in a final volume of 7 μl. The optimal cycling conditions for maximum sensitivity and specificity were 1 minute at 94 °C for initial denaturation, then forty-five cycles of three steps consisting of 0 second at 95 °C, 5 seconds at 60 °C and 9 seconds at 72 °C. Amplification was monitored during each elongation cycle by measuring the level of SYBR® Green I. However, real analysis takes place after PCR. Melting curves are done for each sample and transformation of the melting peak allows determination of Tm. Thus primer-dimer and specific PCR product are discriminated. With this assay, all prominent bacterial contaminants of platelet concentrates listed in Annex XIX and Table 14 were detected. Sensitivity tests were performed on the 9 most frequent bacterial contaminants of platelets. The detection limit was less than 20 genome copies for *E. cloacae*, *B. cereus*, *S. choleraesuis* and *S. marcescens*; less than 15 genome copies for *P. aeruginosa*; and 2 to 3 copies were detected for *S. aureus*, *S.*

epidermidis, *E. coli* and *K. pneumoniae*. Further refinements of assay conditions should increase sensitivity levels.

EXAMPLE 13:

The resolving power of the *tuf* and *atpD* sequences databases is comparable to the biochemical methods for bacterial identification. The present gold standard for bacterial identification is mainly based on key morphological traits and batteries of biochemical tests. Here we demonstrate that the use of *tuf* and *atpD* sequences combined with simple phylogenetic analysis of databases formed by these sequences is comparable to the gold standard. In the process of acquiring data for the *tuf* sequences, we sequenced the *tuf* gene of a strain that was given to us labelled as *Staphylococcus hominis* ATCC 35982. That *tuf* sequence (SEQ ID NO. 192) was incorporated into the *tuf* sequences database and subjected to a basic phylogenic analysis using the Pileup command from version 10 of the GCG package (Genetics Computer Group). This analysis indicated that SEQ ID NO. 192 is not associated with other *S. hominis* strains but rather with the *S. warneri* strains. The ATCC 35982 strain was sent to the reference laboratory of the Laboratoire de santé publique du Québec (LSPQ). They used the classic identification scheme for staphylococci (Kloos and Schleifer, 1975., J. Clin. Microbiol. **1**:82-88). Their results shown that although the colonial morphology could correspond to *S. hominis*, the more precise biochemical assays did not. These assays included discriminant mannitol, mannose and ribose acidification tests as well as rapid and dense growth in deep thioglycolate agar. The LSPQ report identified strain ATCC 35982 as *S. warneri* which confirms our database analysis. The same thing happened for *S. warneri* (SEQ ID NO. 187) which had initially been identified as *S. haemolyticus* by a routine clinical laboratory using a low resolving power automated system (MicroScan, AutoScan-4™). Again, the *tuf* and LSPQ analysis agreed on its identification as *S. warneri*. In numerous other instances, in the course of acquiring *tuf* and *atpD* sequence data from various species and genera,

analysis of our *tuf* and/or *atpD* sequence databases permitted the exact identification of mislabelled or erroneously identified strains. These results clearly demonstrate the usefulness and the high resolving power of our sequence-based identification assays using the *tuf* and *atpD* sequences databases.

EXAMPLE 14:

Detection of group B streptococci from clinical specimens.

Introduction

Streptococcus agalactiae, the group B streptococcus (GBS), is responsible for a severe illness affecting neonate infants. The bacterium is passed from the healthy carrier mother to the baby during delivery. To prevent this infection, it is recommended to treat expectant mothers susceptible of carrying GBS in their vaginal/anal flora. Carrier status is often a transient condition and rigorous monitoring requires cultures and classic bacterial identification weeks before delivery. To improve the detection and identification of GBS we developped a rapid, specific and sensitive PCR test fast enough to be performed right at delivery.

Materials and Methods

GBS clinical specimens. A total of 66 duplicate vaginal/anal swabs were collected from 41 consenting pregnant women admitted for delivery at the Centre Hospitalier Universitaire de Québec, Pavillon Saint-François d'Assise following the CDC recommendations. The samples were obtained either before or after rupture of membranes. The swab samples were tested at the Centre de Recherche en Infectiologie de l'Université Laval within 24 hours of collection. Upon receipt, one swab was cut and then the tip of the swab was added to GNS selective broth for identification of group B streptococci (GBS) by the standard culture methods

recommended by the CDC. The other swab was processed following the instruction of the IDI DNA extraction kit (Infectio Diagnotics (IDI) Inc.) prior to PCR amplification.

Oligonucleotides. PCR primers, Tsag340 (SEQ ID NO. 549) and Tsag552 (SEQ ID NO. 550) complementary to the regions of the *tuf* gene unique for GBS were designed based upon a multiple sequence alignment using our repertory of *tuf* sequences. Oligo primer analysis software (version 5.0) (National Biosciences) was used to analyse primers annealing temperature, secondary structure potential as well as mispriming and dimerization potential. The primers were synthesized using a model 391 DNA synthesizer (Applied Biosystems).

A pair of fluorescently labeled adjacent hybridization probes Sag465-F (SEQ ID NO. 583) and Sag436-C (SEQ ID NO. 582) were synthesized and purified by Operon Technologies. They were designed to meet the recommendations of the manufacturer (Idaho Technology) and based upon multiple sequence alignment analysis using our repertory of *tuf* sequences to be specific and ubiquitous for GBS. These adjacent probes, which are separated by one nucleotide, allow fluorescence resonance energy transfer (FRET), generating an increased fluorescence signal when both hybridized simultaneously to their target sequences. The probe SEQ ID NO. 583 was labeled with FITC in 3 prime while SEQ ID NO. 582 was labeled with Cy5 in 5 prime. The Cy5-labeled probes contained a 3'-blocking phosphate group to prevent extension of the probes during the PCR reactions.

PCR amplification. Conventional amplifications were performed either from 2 μl of a purified genomic DNA preparation or cell lysates of vaginal/anal specimens. The 20 μl PCR mixture contained 0.4 μM of each GBS-specific primer (SEQ ID NOs. 549-550), 200 μM of each deoxyribonucleotide (Pharmacia Biotech), 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 2.5 mM $MgCl_2$, 3.3 mg/ml bovine serum albumin (BSA) (Sigma), and 0.5 U of *Taq* polymerase (Promega) combined with the TaqStart™ antibody (Clontech). The TaqStart™ antibody, which is a neutralizing monoclonal antibody of *Taq* DNA

polymerase, was added to all PCR reactions to enhance the efficiency of the amplification. The PCR mixtures were subjected to thermal cycling (3 min at 95 °C and then 40 cycles of 1 s at 95 °C, and 30 s at 62 °C with a 2-min final extension at 72 °C) with a PTC-200 DNA Engine thermocycler (MJ research). The PCR-amplified reaction mixture was resolved by agarose gel electrophoresis.

The LightCycler™ PCR amplifications were performed with 1 μl of a purified genomic DNA preparation or cell lysates of vaginal/anal specimens. The 10μl amplification mixture consisted of 0.4 μM each GBS-specific primer (SEQ ID NOs. 549-550), 200 μM each dNTP, 0.2 μM each fluorescently labeled probe (SEQ ID NOs. 582-583), 300 μg/ml BSA (Sigma), and 1 μl of 10x PC2 buffer (containing 50 mM Tris-HCl (pH 9.1), 16 mM ammonium sulfate, 3.5 mM Mg^{2+}, and 150 μg/ml BSA) and 0.5 U KlenTaq1™ (AB Peptides) coupled with TaqStart™ antibody (Clontech). KlenTaq1™ is a highly active and more heat-stable DNA polymerase without 5'-exonuclease activity. This prevents hydrolysis of hybridized probes by the 5' to 3' exonuclease activity. A volume of 7 μl of the PCR mixture was transferred into a composite capillary tube (Idaho Technology). The tubes were then centrifuged to move the reaction mixture to the tips of the capillaries and then cleaned with optical-grade methanol. Subsequently the capillaries were loaded into the carousel of a LC32 LightCycler™ (Idaho Technology), an instrument that combines rapid-cycle PCR with fluorescence analysis for continuous monitoring during amplification. The PCR reaction mixtures were subjected to a denaturation step at 94 °C for 3 min followed by 45 cycles of 0 s at 94 °C, 20 s at 64 °C and 10 s at 72 °C with a temperature transition rate of 20 °C/s. Fluorescence signals were obtained at each cycle by sequentially positioning each capillary on the carousel at the focus of optical elements affiliated to the built-in fluorimeter for 100 milliseconds. Complete amplification and analysis required about 35 min.

Specificity and sensitivity tests. The specificity of the conventional and LightCycler™ PCR assays was verified by using purified genomic DNA (0.1 ng/reaction) from a battery of ATCC reference strains representing 35 clinically

relevant gram-positive species (*Abiotrophia defectiva* ATCC 49176, *Bifidobacterium breve* ATCC 15700, *Clostridium difficile* ATCC 9689, *Corynebacterium urealyticum* ATCC 43042, *Enterococcus casseliflavus* ATCC 25788, *Enterococcus durans* ATCC 19432, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium* ATCC 19434, *Enterococcus gallinarum* ATCC 49573, *Enterococcus raffinosus* ATCC 49427, *Lactobacillus reuteri* ATCC 23273, *Lactococcus lactis* ATCC 19435, *Listeria monocytogenes* ATCC 15313, *Peptococcus niger* ATCC 27731, *Peptostreptococcus anaerobius* ATCC 27337, *Peptostreptococcus prevotii* ATCC 9321, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 14990, *Staphylococcus haemolyticus* ATCC 29970, *Staphylococcus saprophyticus* ATCC 15305, *Streptococcus agalactiae* ATCC 27591, *Streptococcus anginosus* ATCC 33397, *Streptococcus bovis* ATCC 33317, *Streptococcus constellatus* ATCC 27823, *Streptococcus dysgalactiae* ATCC 43078, *Streptococcus gordonii* ATCC 10558, *Streptococcus mitis* ATCC 33399, *Streptococcus mutans* ATCC 25175, *Streptococcus oralis* ATCC 35037, *Streptococcus parauberis* ATCC 6631, *Streptococcus pneumoniae* ATCC 6303, *Streptococcus pyogenes* ATCC 19615, *Streptococcus salivarius* ATCC 7073, *Streptococcus sanguinis* ATCC 10556, *Streptococcus uberis* ATCC 19436). These microbial species included 15 species of streptococci and many members of the normal vaginal and anal floras. In addition, 40 GBS isolates of human origin, whose identification was confirmed by a latex agglutination test (Streptex, Murex), were also used to evaluate the ubiquity of the assay.

For determination of the sensitivities (i.e., the minimal number of genome copies that could be detected) for conventional and LightCycler™ PCR assays, serial 10-fold or 2-fold dilutions of purified genomic DNA from 5 GBS ATCC strains were used.

Results

Evaluation of the GBS-specific conventional and LightCycler™ PCR assays. The specificity of the two assays demonstrated that only DNAs from GBS

strains could be amplified. Both PCR assays did not amplify DNAs from any other bacterial species tested including 14 streptococcal species other than GBS as well as phylogenetically related species belonging to the genera *Enterococcus*, *Peptostreptococcus* and *Lactococcus*. Important members of the vaginal or anal flora, including coagulase-negative staphylococci, *Lactobacillus* sp., and *Bacteriodes* sp. were also negative with the GBS-specific PCR assay. The LightCycler™ PCR assays detected only GBS DNA by producing an increased fluorescence signal which was interpreted as a positive PCR result. Both PCR methods were able to amplify all of 40 GBS clinical isolates, showing a perfect correlation with the phenotypic identification methods.

The sensitivity of the assay was determined by using purified genomic DNA from the 5 ATCC strains of GBS. The detection limit for all of these 5 strains was one genome copy of GBS. The detection limit of the assay with the LightCycler™ was 3.5 fg of genomic DNA (corresponding to 1-2 genome copies of GBS). These results confirmed the high sensitivity of our GBS-specific PCR assay.

Direct Detection of GBS from vaginal/anal specimens. Among 66 vaginal/anal specimens tested, 11 were positive for GBS by both culture and PCR. There was one sample positive by culture only. The sensitivity of both PCR methods with vaginal/anal specimens for identifying colonization status in pregnant women at delivery was 91.7% when compared to culture results. The specificity and positive predictive values were both 100% and the negative predictive value was 97.8%. The time for obtaining results was approximately 45 min for LightCycler™ PCR, approximately 100 min for conventional PCR and 48 hours for culture.

Conclusion

We have developed two PCR assays (conventional and LightCycler™) for the detection of GBS, which are specific (i.e., no amplification of DNA from a variety of bacterial species other than GBS) and sensitive (i.e., able to detect around 1

genome copy for several reference ATCC strains of GBS). Both PCR assays are able to detect GBS directly from vaginal/anal specimens in a very short turnaround time. Using the real-time PCR assay on LightCycler™, we can detect GBS carriage in pregnant women at delivery within 45 minutes.

EXAMPLE 15:

Simultaneous detection and identification of *Streptococcus pyogenes* and its pyrogenic exotoxin A. The rapid detection of *Streptococcus pyogenes* and of its pyrogenic exotoxin A is of clinical importance. We developed a multiplex assay which permits the detection of strains of *S. pyogenes* carrying the pyrogenic toxin A gene, which is associated with scarlet fever and other pathologies. In order to specifically detect *S. pyogenes*, nucleotide sequences of the pyrrolidone carboxylyl peptidase (*pcp*) gene were aligned to design PCR primers Spy291 (SEQ ID NO. 1211) and Spy473 (SEQ ID NO. 1210). Next, we designed primers for the specific detection of the pyrogenic exotoxin A. Nucleotide sequences of the *speA* gene, carried on the bacteriophage T12, were aligned as shown in Annex XXIII to design PCR primers Spytx814 (SEQ ID NO. 994) and Spytx 927 (SEQ ID NO. 995).

The primer pairs: oligos SEQ ID NOs. 1210-1211, yielding an amplification product of 207 bp, and oligos SEQ ID NOs. 994-995, yielding an amplification product of 135 bp, were used in a multiplex PCR assay.

PCR amplification was carried out using 0.4 μM of both pairs of primers, 2.5 mM $MgCl_2$, BSA 0.05 μM , dNTP 0.2 μM (Pharmacia), 10mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM $MgCl_2$, 0.5 U *Taq* DNA polymerase (Promega) coupled with TaqStart™ antibody (Clontech Laboratories Inc.), and 1 μl of genomic DNA sample in a final volume of 20 μl. PCR amplification was performed using a PTC-200 thermal cycler (MJ Research). The optimal cycling conditions for maximum specificity and sensitivity were 3 minutes at 94 °C for

initial denaturation, then forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 63 °C, followed by a final step of 2 minutes at 72 °C. Detection of the PCR products was made by electrophoresis in agarose gels (2 %) containing 0.25 μg/ml of ethidium bromide. Visualization of the PCR products was made under UV at 254 nm.

The detection limit was less than 5 genome copies for both *S. pyogenes* and its pyrogenic exotoxin A. The assay was specific for pyrogenic exotoxin A-producing *S. pyogenes*: strains of the 27 other species of *Streptococcus* tested, as well as 20 strains of various gram-positive and gram-negative bacterial species were all negative.

A similar approach was used to design an alternative set of *speA*-specific primers (SEQ ID NOs. 996 to 998, see Annex XXIV). In addition, another set of primers based on the *tuf* gene (SEQ ID NOs. 999 to 1001, see Annex XXV) could be used to specifically detect *Streptococcus pyogenes*.

EXAMPLE 16:

Real-time detection and identification of Shiga toxin-producing bacteria. Shiga toxin-producing *Escherichia coli* and *Shigella dysenteriae* cause bloody diarrhea. Currently, identification relies mainly on the phenotypic identification *of S. dysenteriae* and *E. coli* serotype O157:H7. However, other serotypes of *E. coli* are increasingly found to be producers of type 1 and/or type 2 Shiga toxins. Two pairs of PCR primers targeting highly conserved regions present in each of the Shiga toxin genes stx_1 and stx_2 were designed to amplify all variants of those genes (see Annexes XXVI and XXVII). The first primer pair, oligonucleotides 1SLT224 (SEQ ID NO. 1081) and 1SLT385 (SEQ ID NO. 1080), yields an amplification product of 186 bp from the stx_1 gene. For this amplicon, the 1SLTB1-Fam (SEQ ID NO. 1084) molecular beacon was designed for the specific detection of stx_1

using the fluorescent label 6-carboxy-fluorescein. The 1SltS1-FAM (SEQ ID NO. 2012) molecular scorpion was also designed as an alternate way for the specific detection of *stx_1*. A second pair of PCR primers, oligonucleotides 2SLT537 (SEQ ID NO. 1078) and 2SLT678b (SEQ ID NO. 1079), yields an amplification product of 160 bp from the *stx_2* gene. Molecular beacon 2SLTB1-Tet (SEQ ID NO. 1085) was designed for the specific detection of *stx_2* using the fluorescent label 5-tetrachloro-fluorescein. Both primer pairs were combined in a multiplex PCR assay.

PCR amplification was carried out using 0.8 μM of primer pair SEQ ID NOs. 1080-1081, 0.5 μM of primer pair SEQ ID NOs. 1078-1079, 0.3 μM of each molecular beacon, 8 mM $MgCl_2$, 490 μg/mL BSA, 0.2 mM dNTPs (Pharmacia), 50 mM Tris-HCl, 16 mM NH_4SO_4, 1X TaqMaster (Eppendorf), 2.5 U KlenTaq1 DNA polymerase (AB Peptides) coupled with TaqStart™ antibody (Clontech Laboratories Inc.), and 1 μl of genomic DNA sample in a final volume of 25 μl. PCR amplification was performed using a SmartCycler thermal cycler (Cepheid). The optimal cycling conditions for maximum sensitivity and specificity were 60 seconds at 95 °C for initial denaturation, then 45 cycles of three steps consisting of 10 seconds at 95 °C, 15 seconds at 56 °C and 5 seconds at 72 °C. Detection of the PCR products was made in real-time by measuring the fluorescent signal emitted by the molecular beacon when it hybridizes to its target at the end of the annealing step at 56 °C.

The detection limit was the equivalent of less than 5 genome copies. The assay was specific for the detection of both toxins, as demonstrated by the perfect correlation between PCR results and the phenotypic characterization performed using antibodies specific for each Shiga toxin type. The assay was successfully performed on several Shiga toxin-producing strains isolated from various geographic areas of the world, including 10 O157:H7 *E. coli*, 5 non-O157:H7 *E. coli* and 4 *S. dysenteriae*.

EXAMPLE 17:

Development of a PCR assay for the detection and identification of staphylococci at genus and species levels and its associated *mecA* gene. The *Staphylococcus*-specific PCR primers described in Example 7 (SEQ ID NOs. 553 and 575) were used in multiplex with the *mecA*-specific PCR primers and the *S. aureus*-specific primers described in our assigned US patent no. 5,994,066 (SEQ ID NOs. 261 and 262 for *mecA* and SEQ ID NOs. 152 and 153 for *S.aureus* in the said patent). Sequence alignment analysis of 10 publicly available *mecA* gene sequences allowed to design an internal probe specific to *mecA* (SEQ ID NO. 1177). An internal probe was also designed for the *S. aureus*-specific amplicon (SEQ ID NO 1234). PCR amplification and agarose gel electrophoresis of the amplified products were performed as described in Example 7, with the exception that 0.4 μM (each) of the two *Staphylococcus*-specific primers (SEQ ID NOs. 553 and 575) and 0.4 μM (each) of the *mecA*-specific primers and 0.4 μM (each) of the *S. aureus*-specific primers were used in the PCR mixture. The specificity of the multiplex assay with 40-cycle PCR protocols was verified by using purified genomic DNA from five methicillin-resistant and fifteen methicillin-sensitive staphylococcal strains. The sensitivity of the multiplex assay with 40-cycle PCR protocols was determined by using purified genomic DNA from twenty-three methicillin-resistant and twenty-eight methicillin-sensitive staphylococcal strains. The detection limit was 2 to 10 genome copies of genomic DNA, depending on the staphylococcal species tested. Furthermore, the *mecA*-specific internal probe, the *S. aureus*-specific internal probe and the coagulase-negative staphylococci-specific internal probe (described in Example 7) were able to recognize twenty-three methicillin-resistant staphylococcal strains and twenty-eight methicillin-sensitive staphylococcal strains with high sensitivity and specificity.

The format of the assay is not limited to the one described above. A person skilled in the art could adapt the assay for different formats such as PCR with real-time detection using molecular beacon probes. Molecular beacon probes designed to be used in this assay include, but are not limited to, SEQ ID NO. 1232 for detection of the *S. aureus*-specific amplicon, SEQ ID NO. 1233 for detection of coagulase-negative staphylococci and SEQ ID NO. 1231 for detection of *mecA*.

Alternatively, a multiplex PCR assay containing the *Staphylococcus*-specific PCR primers described in Example 7 (SEQ ID NOs. 553 and 575) and the *mecA*-specific PCR primers described in our assigned US patent no. 5,994,066 (SEQ ID NOs. 261 and 262 in the said patent) were developed. PCR amplification and agarose gel electrophoresis of the amplified products were performed as described in Example 7, with the exception that 0.4 μM (each) of the *Staphylococcus*-specific primers (SEQ ID NOs. 553 and 575) and 0.4 μM (each) of the *mecA*-specific primers described in our assigned US patent no. 5,994,066 (SEQ ID NOs. 261 and 262 in the said patent) were used in the PCR mixture. The sensitivity of the multiplex assay with 40-cycle PCR protocols was determined by using purified genomic DNA from two methicillin-resistant and five methicillin-sensitive staphylococcal strains. The detection limit was 2 to 5 copies of genomic DNA, depending on the staphylococcal species tested. The specificity of the multiplex PCR assay coupled with capture-probe hybridization was tested with two strains of methicillin-resistant *S. aureus*, two strains of methicillin-sensitive *S. aureus* and seven strains of methicillin-sensitive coagulase-negative staphylococci. The *mecA*-specific internal probe (SEQ ID NO. 1177) and the *S. aureus*-specific internal probe (SEQ ID NO. 587) described in Example 7 were able to recognize all the strains with high specificity showing a perfect correlation with susceptibility to methicillin. The sensitivity of the PCR assay coupled with capture-probe hybridization was tested with one strain of methicillin-resistant *S. aureus*. The detection limit was around 10 copies of genomic DNA.

EXAMPLE 18:

Sequencing of *pbp1a*, *pbp2b* and *pbp2x* genes of *Streptoccoccus pneumoniae*.

Penicillin resistance in *Streptococcus pneumoniae* involves the sequential alteration of up to five penicillin-binding proteins (PBPs) 1A, 1B, 2A, 2X and 2B in such a way that their affinity is greatly reduce toward the antibiotic molecule. The altered PBP genes have arisen as the result of interspecies recombination events from related streptococcal species. Among the PBPs usually found in *S. pneumoniae*, PBPs 1A, 2B, and 2X play the most important role in the development of penicillin resistance. Alterations in PBP 2B and 2X mediate low-level resistance to penicillin while additional alterations in PBP 1A plays a significant role in full penicillin resistance.

In order to generate a database for *pbp* sequences that can be used for design of primers and/or probes for the specific and ubiquitous detection of β-lactam resistance in *S. pneumoniae*, *pbp1a*, *pbp2b* and *pbp2x* DNA fragments sequenced by us or selected from public databases (GenBank and EMBL) from a variety of *S. pneumoniae* strains were used to design oligonucleotide primers. This database is essential for the design of specific and ubiquitous primers and/or probes for detection of β-lactam resistance in *S. pneumoniae* since the altered PBP 1A, PBP 2B and PBP 2X of β-lactam resistant *S. pneumoniae* are encoded by mosaic genes with numerous sequence variations among resistant isolates. The PCR primers were located in conserved regions of *pbp* genes and were able to amplify *pbp1a*, *pbp2b*, and *pbp2x* sequences of several strains of *S. pneumoniae* having various levels of resistance to penicillin and third-generation cephalosporins. Using primer pairs SEQ ID NOs. 1125 and 1126, SEQ ID NOs. 1142 and 1143, SEQ ID NOs. 1146 and 1147, it was possible to amplify and determine *pbp1a* sequences SEQ ID NOs. 1004-1018, 1648, 2056-2060 and 2062-2064, *pbp2b* sequences SEQ ID NOs. 1019-1033, and *pbp2x* sequences SEQ ID NOs. 1034-1048. Six other PCR primers

(SEQ ID NOs. 1127-1128, 1144-1145, 1148-1149) were also designed and used to complete the sequencing of *pbp1a, pbp2b* and *pbp2x* amplification products. The described primers (SEQ ID NOs. 1125 and 1126, SEQ ID NOs. 1142 and 1143, SEQ ID NOs. 1146 and 1147, SEQ ID NOs. 1127-1128, 1144-1145, 1148-1149) represent a powerful tool for generating new *pbp* sequences for design of primers and/or probes for detection of β-lactam resistance in *S. pneumoniae*.

EXAMPLE 19:

Sequencing of *hexA* genes of *Streptococcus* species. The *hexA* sequence of *S. pneumoniae* described in our assigned US patent no. 5,994,066 (SEQ ID NO. 31 in the said patent, SEQ ID NO. 1183 in the present application) allowed the design of a PCR primer (SEQ ID NO. 1182) which was used with primer Spn1401 described in our assigned US patent no. 5,994,066 (SEQ ID NO. 156 in the said patent, SEQ ID NO. 1179 in the present application) to generate a database for *hexA* sequences that can be used to design primers and/or probes for the specific identification and detection of *S. pneumoniae* (Annex XLII). Using primers SEQ ID NO. 1179 and SEQ ID NO. 1182 (Annex XLII), it was possible to amplify and determine the *hexA* sequence from *S. pneumoniae* (4 strains) (SEQ ID NOs. 1184-1187), *S. mitis* (three strains) (SEQ ID NOs. 1189-1191) and *S. oralis* (SEQ ID NO. 1188).

EXAMPLE 20:

Development of multiplex PCR assays coupled with capture probe hybridization for the detection and identification of *Streptococcus pneumoniae* and its penicillin resistance genes.

Two different assays were developed to identify *S. pneumoniae* and its susceptibility to penicillin.

ASSAY I:

Bacterial strains. The specificity of the multiplex PCR assay was verified by using a panel of ATCC (American Type Culture Collection) reference strains consisting of 33 gram-negative and 67 gram-positive bacterial species (Table 13). In addition, a total of 98 strains of *S. pneumoniae*, 16 strains of *S. mitis* and 3 strains of *S. oralis* from the American Type Culture Collection, the microbiology laboratory of the Centre Hospitalier Universitaire de Québec, Pavillon Centre Hospitalier de l'Université Laval (CHUL), (Ste-Foy, Québec, Canada), the Laboratoire de santé publique du Québec, (Sainte-Anne-de-Bellevue, Québec, Canada), the Sunnybrook and Women's College Health Sciences Centre (Toronto, Canada), the Infectious Diseases Section, Department of Veterans Affairs Medical Center, (Houston, USA) were also tested to further validate the *Streptococcus pneumoniae*-specific PCR assay. The penicillin MICs (minimal inhibitory concentrations) were measured by the broth dilution method according to the recommended protocol of NCCLS.

PCR primers and internal probes. The analysis of *hexA* sequences from a variety of streptococcal species from the publicly avalaible *hexA* sequence and from the database described in Example 19 (SEQ ID NOs. 1184-1191) allowed the selection of a PCR primer specific to *S. pneumoniae,* SEQ ID NO. 1181. This primer was used with the *S. pneumoniae*-specific primer SEQ ID NO. 1179 to generate an amplification product of 241 bp (Annex XLII). The PCR primer SEQ ID NO. 1181 is located 127 nucleotides downstream on the *hexA* sequence compared to the original *S. pneumoniae*-specific PCR primer Spn1515 described in our assigned US patent no. 5,994,066 (SEQ ID NO. 157 in the said patent). These modifications were done to ensure the design of the *S. pneumoniae*-specific internal probe according to the new *hexA* sequences of several streptococcal species from the database described in Example 19 (SEQ ID NOs. 1184-1191).

The analysis of *pbp1a* sequences from *S. pneumoniae* strains with various levels of penicillin resistance from public databases and from the database described in Example 18 allowed the identification of amino acid substitutions Ile-459 to Met and Ser-462 to Ala that occur in isolates with high-level penicillin resistance (MICs ≥ 1μg/ml), and amino acid substitutions Ser-575 to Thr, Gln-576 to Gly and Phe-577 to Tyr that are common to all penicillin-resistant isolates with MICs ≥ 0.25 μg/ml. As shown in Annex XXXI, PCR primer pair SEQ ID NOs. 1130 and 1131 were designed to detect high-level penicillin resistance (MICs ≥ 1μg/ml), whereas PCR primer pair SEQ ID NOs. 1129 and 1131 were designed to detect intermediate- and high-level penicillin resistance (MICs ≥ 0.25μg/ml).

The analysis of *hexA* sequences from the publicly avalaible *hexA* sequence and from the database described in Example 19 allowed the design of an internal probe specific to *S. pneumoniae* (SEQ ID NO. 1180) (Annex XLII). The range of mismatches between the *S. pneumoniae*-specific 241-bp amplicon was from 2 to 5, in the middle of the 19-bp probe. The analysis of *pbp1a* sequences from public databases and from the database described in Example 18 allowed the design of five internal probes containing all possible mutations to detect the high-level penicillin resistance 383-bp amplicon (SEQ ID NOs. 1197, 1217-1220). Alternatively, two other internal probes (SEQ ID NOs. 2024-2025) can also be used to detect the high-level penicillin resistance 383-bp amplicon. Five internal probes containing all possible mutations to detect the 157-bp amplicon which includes intermediate- and high-level penicillin resistance were also designed (SEQ ID NOs. 1094, 1192-1193, 1214 and 1216). Design and synthesis of primers and probes, and detection of the probe hybridization were performed as described in Example 7. Annex XXXI illustrates one of the internal probe for detection of the high-level penicillin resistance 383-bp amplicon (SEQ ID NO. 1197) and one of the internal probe for detection of the intermediate- and high-level penicillin resistance 157-bp amplicon (SEQ ID NO. 1193).

PCR amplification. For all bacterial species, amplification was performed from purified genomic DNA using a PTC-200 thermocycler (MJ Research). 1 μl of genomic DNA at 0.1 ng/μl, or 1 μl of a bacterial lysate, was transferred to a 19 μl PCR mixture. Each PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (H 9.0), 0.1% Triton X-100, 2.5 mM $MgCl_2$, 0.1 μM (each) of the *S. pneumoniae*-specific primers SEQ ID NO. 1179 and SEQ ID NO. 1181, 0.2 μM of primer SEQ ID NO. 1129, 0.7 μM of primer SEQ ID NO. 1131, and 0.6 μM of primer SEQ ID NO. 1130, 0.05 mM bovine serum albumin (BSA), and 0.5 U *Taq* polymerase (Promega) coupled with TaqStart^{TM} antibody. In order to generate Digoxigenin (DIG)-labeled amplicons for capture probe hybridization, 0.1X PCR DIG labeling four deoxynucleoside triphosphates mix (Boehringer Mannheim GmbH) was used for amplification.

For determination of the sensitivitiy of the PCR assays, 10-fold dilutions of purified genomic DNA were used to determine the minimal number of genome copies which can be detected.

Capture probe hybridization. The DIG–labeled amplicons were hybridized to the capture probes bound to 96-well plates. The plates were incubated with anti-DIG-alkaline phosphatase and the chemiluminescence was measured by using a luminometer (MLX, Dynex Technologies Inc.) after incubation with CSPD and recorded as Relative Light Unit (RLU). The RLU ratio of tested sample with and without captures probes was then calculated. A ratio $\geq$ 2.0 was defined as a positive hybridization signal. All reactions were performed in duplicate.

Results

Amplifications with the multiplex PCR assay. The specificity of the assay was assessed by performing 40-cycle PCR amplifications with the panel of gram-positive (67 species from 12 genera) and gram-negative (33 species from 17

genera) bacterial species listed in Table 13. All bacterial species tested other than *S. pneumoniae* were negative except *S. mitis* and *S. oralis*. Ubiquity tests were performed using a collection of 98 *S. pneumoniae* strains including high-level penicillin resistance (n=53), intermediate resistance (n=12) and sensitive (n=33) strains. There was a perfect correlation between PCR and standard susceptibility testing for 33 penicillin-sensitive isolates. Among 12 *S. pneumoniae* isolates with intermediate penicillin resistance based on susceptibility testing, 11 had intermediate resistance based on PCR, but one *S. pneumoniae* isolate with penicillin MIC of 0.25 μg/ml showed a high-level penicillin resistance based on genotyping. Among 53 isolates with high-level penicillin resistance based on susceptibility testing, 51 had high-level penicillin resistance based on PCR but two isolates with penicillin MIC > 1 μg/ml showed an intermediate penicillin resistance based on genotyping. In general, there was a good correlation between the genotype and classical culture method for bacterial identification and susceptibility testing.

The sensitivity of the *S. pneumoniae*-specific assay with 40-cycle PCR protocols was determined by using purified genomic DNA from 9 isolates of *S. pneumoniae*. The detection limit was around 10 copies of genomic DNA for all of them.

Post-PCR hybridization with internal probes. The specificity of the multiplex PCR assay coupled with capture-probe hybridization was tested with 98 strains of *S. pneumoniae*, 16 strains of *S. mitis* and 3 strains of *S. oralis*. The internal probe specific to *S. pneumoniae* (SEQ ID NO. 1180) detected all 98 *S. pneunoniae* strains but did not hybridize to the *S. mitis* and *S. oralis* amplicons. The five internal probes specific to the high-level resistance amplicon (SEQ ID NOs. 1197, 1217-1220) detected all amplification patterns corresponding to high-level resistance. The two *S. pneumoniae* strains with penicillin MIC > 1 μg/ml that showed an intermediate penicillin resistance based on PCR amplification were also intermediate resistance based on probe hybridization. Similarly, among 12 strains

with intermediate-penicillin resistance based on susceptibility testing, 11 showed intermediate-penicillin resistance based on hybridization with the five internal probes specific to the intermediate and high-level resistance amplicon (SEQ ID NOs. 1094, 1192-1193, 1214 and 1216). The strain described above having a penicillin MIC of 0.25 μg/ml which was high-level penicillin resistance based on PCR amplification was also high-level resistance based on probe hybridization. In summary, the combination of the multiplex PCR and hybridization assays results in a highly specific test for the detection of penicillin-resistant *Streptococcus pneumoniae*.

ASSAY II:

Bacterial strains. The specificity of the multiplex PCR assay was verified by using the same strains as those used for the development of Assay I. The penicillin MICs (minimal inhibitory concentrations) were measured by the broth dilution method according to the recommended protocol of NCCLS.

PCR primers and internal probes. The analysis of *pbp1a* sequences from *S. pneumoniae* strains with various levels of penicillin resistance from public databases and from the database described in Example 18 allowed the design of two primers located in the constant region of *pbp1a*. PCR primer pair (SEQ ID NOs. 2015 and 2016) was designed to amplify a 888-bp variable region of *pbp1a* from all *S. pneumoniae* strains. A series of internal probes were designed for identification of the *pbp1a* mutations associated with penicillin resistance in *S. pneumoniae*. For detection of high-level penicillin resistance (MICs ≥ 1μg/ml), three internal probes were designed (SEQ ID NOs. 2017-2019). Alternaltively, ten other internal probes were designed that can also be used for detection of high-level resistance within the 888-bp *pbp1a* amplicon: (1) three internal probes for identification of the amino acid substitutions Thr-371 to Ser or Ala within the motif S370TMK (SEQ ID NOs. 2031-2033); (2) two internal probes for detection

of the amino acid substitutions Ile-459 to Met and Ser-462 to Ala near the motif S428RN (SEQ ID NOs. 1135 and 2026); (3) two internal probes for identification of the amino acid substitutions Asn-443 to Asp (SEQ ID NOs. 1134 and 2027); and (4) three internal probes for detection of all sequence variations within another region (SEQ ID NOs. 2028-2030). For detection of high-level and intermediate penicillin resistance (MICs ≥ 0.25 μg/ml), four internal probes were designed (SEQ ID NOs. 2020-2023). Alternatively, six other internal probes were designed for detection of the four consecutive amino acid substitutions T574SQF to A574TGY near the motif K557TG (SEQ ID NOs. 2034-2039) that can also be used for detection of intermediate- and high-level resistance within the 888-bp *pbp1a* amplicon.

PCR amplification. For all bacterial species, amplification was performed from purified genomic DNA using a PTC-200 thermocycler (MJ Research). 1 μl of genomic DNA at 0.1 ng/μl, or 1 μl of a bacterial lysate, was transferred to a 19 μl PCR mixture. Each PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM $MgCl_2$, 0.08 μM (each) of the *S. pneumoniae*-specific primers SEQ ID NO. 1179 and SEQ ID NO. 1181, 0.4 μM of the *pbp1a*-specific primer SEQ ID NO. 2015, 1.2 μM of *pbp1a*-specific primer SEQ ID NO. 2016, 0.05 mM bovine serum albumin (BSA), and 0.5 U *Taq* polymerase (Promega) coupled with TaqStart™ antibody. In order to generate Digoxigenin (DIG)-labeled amplicons for capture probe hybridization, 0.1X PCR DIG labeling four deoxynucleoside triphosphates mix (Boehringer Mannheim GmbH) was used for amplification.

For determination of the sensitivities of the PCR assays, 10-fold dilutions of purified genomic DNA were used to determine the minimal number of genome copies which can be detected.

Capture probe hybridization. The DIG–labeled amplicons were hybridized to the capture probes bound to 96-well plates as described for Assay I.

Results

Amplifications with the multiplex PCR assay. The specificity of the assay was assessed by performing 40-cycle PCR amplifications with the panel of gram-positive (67 species from 12 genera) and gram-negative (33 species from 17 genera) bacterial species listed in Table 13. All bacterial species tested other than *S. pneumoniae* were negative except *S. mitis* and *S. oralis*. Ubiquity tests were performed using a collection of 98 *S. pneumoniae* strains including high-level penicillin resistance (n=53), intermediate resistance (n=12) and sensitive (n=33) strains. All the above *S. pneumoniae* strains produced the 888-bp amplicon corresponding to *pbp1a* and the 241-bp fragment corresponding to *hexA*.

The sensitivity of the *S. pneumoniae*-specific assay with 40-cycle PCR protocols was determined by using purified genomic DNA from 9 isolates of *S. pneumoniae*. The detection limit was around 10 copies of genomic DNA for all of them.

Post-PCR hybridization with internal probes. The specificity of the multiplex PCR assay coupled with capture-probe hybridization was tested with 98 strains of *S. pneumoniae*, 16 strains of *S. mitis* and 3 strains of *S. oralis*. The internal probe specific to *S. pneumoniae* (SEQ ID NO. 1180) detected all 98 *S. pneunoniae* strains but did not hybridize to the *S. mitis* and *S. oralis* amplicons. The three internal probes (SEQ ID NOs 2017-2019) specific to high-level resistance detected all the 43 strains with high-level penicillin resistance based on susceptibility testing. Among 12 isolates with intermediate-penicillin resistance based on susceptibility testing, 11 showed intermediate-penicillin resistance based on hybridization with 4 internal probes (SEQ ID NOs. 2020-2023) and one strain

having penicillin MIC of 0.25 µg/ml was misclassified as high-level penicillin resistance. In summary, the combination of the multiplex PCR and hybridization assays results in a highly specific test for the detection of penicillin-resistant *Streptococcus pneumoniae*.

EXAMPLE 21:

Sequencing of the vancomycin resistance *vanA, vanC1, vanC2* and *vanC3* genes. The publicly available sequences of the *vanH-vanA-vanX-vanY* locus of transposon Tn1546 from *E. faecalis*, *vanC1* sequence from one strain of *E. gallinarum*, *vanC2* and *vanC3* sequences from a variety of *E. casseliflavus* and *E. flavescens* strains, respectively, allowed the design of PCR primers able to amplify the *vanA, vanC1, vanC2* and *vanC3* sequences of several *Enterococcus* species. Using primer pairs van6877 and van9106 (SEQ ID NOs. 1150 and 1155), vanC1-122 and vanC1-1315 (SEQ ID NOs. 1110 and 1109), and vanC2C3-1 and vanC2C3-1064 (SEQ ID NOs. 1108 and 1107), it was possible to amplify and determine *vanA* sequences SEQ ID NOs. 1049-1057, *vanC1* sequences SEQ ID NOs. 1058-1059, *vanC2* sequences SEQ ID NOs. 1060-1063 and *vanC3* sequences SEQ ID NOs. 1064-1066, respectively. Four other PCR primers (SEQ ID NOs. 1151-1154) were also designed and used to complete the sequencing of *vanA* amplification products.

EXAMPLE 22:

Development of a PCR assay for the detection and identification of enterococci at genus and species levels and its associated resistance genes *vanA* and *vanB*. The comparison of *vanA* and *vanB* sequences revealed conserved regions allowing the design of PCR primers specific to both *vanA* and *vanB* sequences (Annex XXXVIII). The PCR primer pair vanAB459 and vanAB830R (SEQ ID NOs. 1112 and 1111) was used in multiplex with the *Enterococcus*-specific primers Encg313dF and Encg599c (SEQ ID NOs. 1137 and 1136) described in Example

11. Sequence alignment analysis of *vanA* and *vanB* sequences revealed regions suitable for the design of internal probes specific to *vanA* (SEQ ID NO. 1170) and *vanB* (SEQ ID NO. 1171). PCR amplification and agarose gel electropheresis of the amplified products were performed as described in Example 11. The optimal cycling conditions for maximum sensitivity and specificity were found to be 3 min. at 94 °C, followed by forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 62°C, plus a terminal extension at 72 °C for 2 minutes. The specificity of the multiplex assay with 40-cycle PCR was verified by using 0.1 nanogram of purified genomic DNA from a panel of bacteria listed in Table 10. The sensitivity of the multiplex assay with 40-cycle PCR was verified with three strains of *E. casseliflavus*, eight strains of *E. gallinarum*, two strains of *E. flavescens*, two vancomycin-resistant strains of *E. faecalis* and one vancomycin-sensitive strain of *E. faecalis*, three vancomycin-resistant strains of *E. faecium*, one vancomycin-sensitive strain of *E. faecium* and one strain of each of the other enterococcal species listed in Table 10. The detection limit was 1 to 10 copies of genomic DNA, depending on the enterococcal species tested. The *vanA*- and *vanB*-specific internal probes (SEQ ID NOs. 1170 and 1171), as well as the *E. faecalis*- and *E. faecium*-specific internal probes (SEQ ID NOs. 1174 and 602) and the internal probe specific to the group including *E. casseliflavus*, *E. gallinarum* and *E. flavescens* (SEQ ID NO. 1122) described in Example 11, were able to recognize vancomycin-resistant enterococcal species with high sensitivity, specificity and ubiquity showing a perfect correlation between the genotypic and phenotypic analysis.

The format of the assay is not limited to the one described above. A person skilled in the art could adapt the assay for different formats such as PCR with real-time detection using molecular beacon probes. Molecular beacon probes designed to be used in this assay include, but are not limited to, SEQ ID NO. 1236 for the detection of *E. faecalis*, SEQ ID NO. 1235 for the detection of *E. faecium*, SEQ ID NO. 1240 for the detection of *vanA*, and SEQ ID NO. 1241 for the detection of *vanB*.

EXAMPLE 23:

Development of a multiplex PCR assay for detection and identification of vancomycin-resistant *Enterococcus faecalis*, *Enterococcus faecium* and the group including *Enterococcus gallinarum*, *Enterococcus casseliflavus*, and *Enterococcus flavescens*. The analysis of *vanA* and *vanB* sequences revealed conserved regions allowing design of a PCR primer pair (SEQ ID NOs. 1089 and 1090) specific to *vanA* sequences (Annex XXVIII) and a PCR primer pair (SEQ ID NOs. 1095 and 1096) specific to *vanB* sequences (Annex XXIX). The *vanA*-specific PCR primer pair (SEQ ID NOs. 1089 and 1090) was used in multiplex with the *vanB*-specific PCR primer pair described in our assigned US patent 5,994,066 (SEQ ID NOs. 1095 and 1096 in the present patent and SEQ ID NOs. 231 and 232 in the said patent). The comparison of *vanC1*, *vanC2* and *vanC3* sequences revealed conserved regions allowing design of PCR primers (SEQ ID NOs. 1101 and 1102) able to generate a 158-bp amplicon specific to the group including *E. gallinarum*, *E. casseliflavus* and *E. flavescens* (Annex XXX). The *vanC*-specific PCR primer pair (SEQ ID NOs. 1101 and 1102) was used in multiplex with the *E. faecalis*-specific PCR primer pair described in our assigned US patent 5,994,066 (SEQ ID NOs. 40 and 41 in the said patent) and with the *E. faecium*-specific PCR primer pair described in our patent publication WO98/20157 (SEQ ID NOs. 1 and 2 in the said publication). For both multiplexes, the optimal cycling conditions for maximum sensitivity and specificity were found to be 3 min. at 94 °C, followed by forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 58 °C, plus a terminal extension at 72 °C for 2 minutes. Detection of the PCR products was made by electrophoresis in agarose gels (2 %) containing 0.25 μg/ml of ethidium bromide. The *vanA*-specific PCR primer pair (SEQ ID NOs. 1089 and 1090), the *vanB*-specific primer pair (SEQ ID NOs. 1095 and 1096) and the *vanC*-specific primer pair (SEQ ID NOs. 1101 and 1102) were tested for their specificity by using 0.1 nanogram of purified genomic DNA from a panel of 5 vancomycin-

sensitive *Enterococcus* species, 3 vancomycin-resistant *Enterococcus* species, 13 other gram-positive bacteria and one gram-negative bacterium. Specificity tests were performed with the *E. faecium*-specific PCR primer pair described in our patent publication WO98/20157 (SEQ ID NOs. 1 and 2 in the said publication) and with the *E. faecalis*-specific PCR primer pair described in our assigned US patent 5,994,066 (SEQ ID NOs. 40 and 41 in the said patent) on a panel of 37 gram-positive bacterial species. All *Enterococcus* strains were amplified with high specificity showing a perfect correlation between the genotypic and phenotypic analysis. The sensitivity of the assays was determined for several strains of *E. gallinarum*, *E. casseliflavus*, *E. flavescens* and vancomycin-resistant *E. faecalis* and *E. faecium*. Using each of the *E. faecalis*- and *E. faecium*-specific PCR primer pairs as well as *vanA*-, *vanB*- and *vanC*-specific PCR primers used alone or in multiplex as described above, the sensitivity ranged from 1 to 10 copies of genomic DNA.

The format of the assay is not limited to the one described above. A person skilled in the art could adapt the assay for different formats such as PCR with real-time detection using molecular beacon probes. Molecular beacon probes designed to be used in this assay include, but are not limited to, SEQ ID NO. 1238 for the detection of *E. faecalis*, SEQ ID NO. 1237 for the detection of *E. faecium*, SEQ ID NO. 1239 for the detection of *vanA*, and SEQ ID NO. 1241 for the detection of *vanB*.

Alternatively, another PCR assay was developed for the detection of vancomycin-resistant *E. faecium* and vancomycin-resistant *E. faecalis*. This assay included two multiplex: (1) the first multiplex contained the *vanA*-specific primer pair (SEQ ID NOs. 1090-1091) and the *vanB*-specific PCR primer pair described in our assigned US patent 5,994,066 (SEQ ID NOs. 1095 and 1096 in the present patent and SEQ ID NOs. 231 and 232 in the said patent), and (2) the second multiplex contained the *E. faecalis*-specific PCR primer pair described in our assigned US patent 5,994,066 (SEQ ID NOs. 40 and 41 in the said patent) and the *E. faecium*-specific PCR primer pair described in our patent publication WO98/20157 (SEQ ID NOs. 1

and 2 in the said publication). For both multiplexes, the optimal cycling conditions for maximum sensitivity and specificity were found to be 3 min. at 94 °C, followed by forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 58 °C, plus a terminal extension at 72 °C for 2 minutes. Detection of the PCR products was made by electrophoresis in agarose gels (2 %) containing 0.25 μg/ml of ethidium bromide. The two multiplexes were tested for their specificity by using 0.1 nanogram of purified genomic DNA from a panel of two vancomycin-sensitive *E. faecalis* strains, two vancomycin-resistant *E. faecalis* strains, two vancomycin-sensitive *E. faecium* strains, two vancomycin-resistant *E. faecium* strains, 16 other enterococcal species and 31 other gram-positive bacterial species. All the *E. faecium* and *E. faecalis* strains were amplified with high specificty showing a perfect correlation between the genotypic analysis and the susceptibility to glycopeptide antibiotics (vancomycin and teicoplanin). The sensitivity of the assay was determined for two vancomycin-resistant *E. faecalis* strains and two vancomycin-resistant *E. faecium* strains. The detection limit was 5 copies of genomic DNA for all the strains.

This multiplex PCR assay was coupled with capture-probe hybridization. Four internal probes were designed: one specific to the *vanA* amplicon (SEQ ID NO. 2292), one specific to the *vanB* amplicon (SEQ ID NO. 2294), one specific to the *E. faecalis* amplicon (SEQ ID NO. 2291) and one specific to the *E. faecium* amplicon (SEQ ID NO. 2287). Each of the internal probes detected their specific amplicons with high specificity and sensitivity.

EXAMPLE 24:

Universal amplification involving the EF-G (*fusA*) subdivision of *tuf* sequences. As shown in Figure 3, primers SEQ ID NOs. 1228 and 1229 were designed to amplify the region between the end of *fusA* and the beginning of *tuf* genes in the *str* operon. Genomic DNAs from a panel of 35 strains were tested for PCR amplification with those primers. In the initial experiment, the following strains showed a positive

result: *Abiotrophia adiacens* ATCC 49175, *Abiotrophia defectiva* ATCC 49176, *Bacillus subtilis* ATCC 27370, *Closridium difficile* ATCC 9689, *Enterococcus avium* ATCC 14025, *Enterococcus casseliflavus* ATCC 25788, *Enterococcus cecorum* ATCC 43198, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium* ATCC 19434, *Enterococcus flavescens* ATCC 49996, *Enterococcus gallinarum* ATCC 49573, *Enterococcus solitarius* ATCC 49428, *Escherichia coli* ATCC 11775, *Haemophilus influenzae* ATCC 9006, *Lactobacillus acidophilus* ATCC 4356, *Peptococcus niger* ATCC 27731, *Proteus mirabilis* ATCC 25933, *Staphylococcus aureus* ATCC 43300, *Staphylococcus auricularis* ATCC 33753, *Staphylococcus capitis* ATCC 27840, *Staphylococcus epidemidis* ATCC 14990, *Staphylococcus haemolyticus* ATCC 29970, *Staphylococcus hominis* ATCC 27844, *Staphylococcus lugdunensis* ATCC 43809, *Staphylococcus saprophyticus* ATCC 15305, *Staphylococcus simulans* ATCC 27848, and *Staphylococcus warneri* ATCC 27836. This primer pair could amplify additional bacterial species; however, there was no amplification for some species, suggesting that the PCR cycling conditions could be optimized or the primers modified. For example, SEQ ID NO. 1227 was designed to amplify a broader range of species.

In addition to other possible primer combinations to amplify the region covering *fusA* and *tuf*, Figure 3 illustrates the positions of amplification primers SEQ ID NOs. 1221-1227 which could be used for universal amplification of *fusA* segments. All of the above mentioned primers (SEQ ID NOs. 1221-1229) could be useful for the universal and/or the specific detection of bacteria.

Moreover, different combinations of primers SEQ ID NOs. 1221-1229, sometimes in combination with *tuf* sequencing primer SEQ ID NO. 697, were used to sequence portions of the *str* operon, including the intergenic region. In this manner, the following sequences were generated: SEQ ID NOs. 1518-1526, 1578-1580, 1786-1821, 1822-1834, 1838-1843, 2184, 2187, 2188, 2214-2249, and 2255-2269.

EXAMPLE 25:

<u>DNA fragment isolation from *Staphylococcus saprophyticus* by arbitrarily primed PCR.</u> DNA sequences of unknown coding potential for the species-specific detection and identification of *Staphylococcus saprophyticus* were obtained by the method of arbitrarily primed PCR (AP-PCR).

AP-PCR is a method which can be used to generate specific DNA probes for microorganisms (Fani *et al.*, 1993, Molecular Ecology 2:243-250). A description of the AP-PCR protocol used to isolate a species-specific genomic DNA fragment from *Staphylococcus saprophyticus* follows. Twenty different oligonucleotide primers of 10 nucleotides in length (all included in the AP-PCR kit OPAD (Operon Technologies, Inc., Alameda, CA)) were tested systematically with DNAs from 5 bacterial strains of *Staphylococcus saprophyticus* as well as with bacterial strains of 27 other staphylococcal (non-*S. saprophyticus*) species. For all bacterial species, amplification was performed directly from one μL (0.1 ng/μL) of purified genomic DNA. The 25 μL PCR reaction mixture contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM $MgCl_2$, 1.2 μM of only one of the 20 different AP-PCR primers OPAD, 200 μM of each of the four dNTPs, 0.5 U of *Taq* DNA polymerase (Promega Corp., Madison, Wis.) coupled with TaqStart™ antibody (Clontech Laboratories Inc., Palo Alto, CA). PCR reactions were subjected to cycling using a MJ Research PTC-200 thermal cycler as follows: 3 min at 96 °C followed by 42 cycles of 1 min at 94 °C for the denaturation step, 1 min at 31 °C for the annealing step and 2 min at 72 °C for the extension step. A final extension step of 7 min at 72 °C followed the 42 cycles to ensure complete extension of PCR products. Subsequently, twenty microliters of the PCR-amplified mixture were resolved by electrophoresis on a 1.5 % agarose gel containing 0.25 μg/ml of ethidium bromide. The size of the amplification products was estimated by comparison with a 50-bp molecular weight ladder.

Amplification patterns specific for *Staphylococcus saprophyticus* were observed with the AP-PCR primer OPAD-16 (sequence: 5'-AACGGGCGTC-3'). Amplification with this primer consistently showed a band corresponding to a

DNA fragment of approximately 380 bp for all *Staphylococcus saprophyticus* strains tested but not for any of the other staphylococcal species tested.

The band corresponding to the 380 bp amplicon, specific and ubiquitous for *S. saprophyticus* based on AP-PCR, was excised from the agarose gel and purified using the QIAquick™ gel extraction kit (QIAGEN Inc.). The gel-purified DNA fragment was cloned into the T/A cloning site of the pCR 2.1™ plasmid vector (Invitrogen Inc.) using T4 DNA ligase (New England BioLabs). Recombinant plasmids were transformed into *E. coli* DH5α competent cells using standard procedures. All reactions were performed according to the manufacturer's instructions. Plasmid DNA isolation was done by the method of Birnboim and Doly (Nucleic Acid Res., 1979, 7:1513-1523) for small-scale preparations. All plasmid DNA preparations were digested with the EcoRI restriction endonuclease to ensure the presence of the approximately 380 bp AP-PCR insert into the plasmid. Subsequently, a large-scale and highly purified plasmid DNA preparation was performed from two selected clones shown to carry the AP-PCR insert by using the QIAGEN plasmid purification kit (midi format). These large-scale plasmid preparations were used for automated DNA sequencing.

The 380 bp nucleotide sequence was determined for three strains of *S. saprophyticus* (SEQ ID NOs. 74, 1093, and 1198). Both strands of the AP-PCR insert from the two selected clones were sequenced by the dideoxynucleotide chain termination sequencing method with SP6 and T7 sequencing primers by using the Applied Biosystems automated DNA sequencer (model 373A) with their PRISM™ Sequenase^{RTM} Terminator Double-stranded DNA Sequencing Kit (Applied Biosystems, Foster City, CA).

Optimal species-specific amplification primers (SEQ ID NOs. 1208 and 1209) have been selected from the sequenced AP-PCR *Staphylococcus saprophyticus* DNA fragments with the help of the primer analysis software Oligo™ 5.0 (National BioSciences Inc.). The selected primers were tested in PCR assays to verify their specificity and ubiquity. Data obtained with DNA preparations from reference ATCC strains of 49 gram-positive and 31 gram-negative bacterial

species, including 28 different staphylococcal species, indicate that the selected primer pairs are specific for *Staphylococcus saprophyticus* since no amplification signal has been observed with DNAs from the other staphylococcal or bacterial species tested. This assay was able to amplify efficiently DNA from all 60 strains of *S. saprophyticus* from various origins tested. The sensitivity level achieved for three *S. saprophyticus* reference ATCC strains was around 6 genome copies.

EXAMPLE 26:

Sequencing of prokaryotic *tuf* gene fragments. The comparison of publicly available *tuf* sequences from a variety of bacterial species revealed conserved regions, allowing the design of PCR primers able to amplify *tuf* sequences from a wide range of bacterial species. Using primer pair SEQ ID NOs. 664 and 697, it was possible to amplify and determine *tuf* sequences SEQ ID NOs.: 1-73, 75-241, 607-618, 621, 662, 675, 717-736, 868-888, 932, 967-989, 992, 1002, 1572-1575, 1662-1663, 1715-1733, 1835-1837, 1877-1878, 1880-1881, 2183, 2185, 2200, 2201, and 2270-2272.

EXAMPLE 27:

Sequencing of procaryotic *recA* gene fragments. The comparison of publicly available *recA* sequences from a variety of bacterial species revealed conserved regions, allowing the design of PCR primers able to amplify *recA* sequences from a wide range of bacterial species. Using primer pairs SEQ ID NOs. 921-922 and 1605-1606, it was possible to amplify and determine *recA* sequences SEQ ID NOs.: 990-991, 1003, 1288-1289, 1714, 1756-1763, 1866-1873 and 2202-2212.

EXAMPLE 28:

<u>Specific detection and identification of *Escherichia coli/Shigella* sp. using *tuf* sequences.</u> The analysis of *tuf* sequences from a variety of bacterial species allowed the selection of PCR primers (SEQ ID NOs. 1661 and 1665) and of an internal probe (SEQ ID NO. 2168) specific to *Escherichia coli/Shigella* sp. The strategy used to design the PCR primers was based on the analysis of a multiple sequence alignment of various *tuf* sequences. The multiple sequence alignment included the *tuf* sequences of *Escherichia coli/Shigella* sp. as well as *tuf* sequences from other species and bacterial genera, especially representatives of closely related species. A careful analysis of this alignment allowed the selection of oligonucleotide sequences which are conserved within the target species but which discriminate sequences from other species, especially from the closely related species, thereby permitting the species-specific and ubiquitous detection and identification of the target bacterial species.

The chosen primer pair, oligos SEQ ID NOs. 1661 and 1665, gives an amplification product of 219 bp. Standard PCR was carried out using 0.4 μM of each primer, 2.5 mM $MgCl_2$, BSA 0.05 mM, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1 % Triton X-100, dNTPs 0.2 mM (Pharmacia), 0,5 U *Taq* DNA polymerase (Promega) coupled with TaqStart™ antibody (Clontech Laboratories Inc.), 1 μl of genomic DNA sample in a final volume of 20 μl using a PTC-200 thermocycler (MJ Research). The optimal cycling conditions for maximum sensitivity and specificity were 3 minutes at 95 °C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 60 °C, followed by terminal extension at 72 °C for 2 minutes. Detection of the PCR products was made by electrophoresis in agarose gels (2 %) containing 0.25 μg/ml of ethidium bromide. Visualization of the PCR products was made under UV at 254 nm.

Specificity of the assay was tested by adding to the PCR reactions 0.1 ng of genomic DNA from each of the following bacterial species: *Escherichia coli* (7

strains), *Shigella sonnei, Shigella flexneri, Shigella dysenteriae, Salmonella typhimyurium, Salmonella typhi, Salmonella enteritidis, Tatumella ptyseos, Klebsiella pneumoniae* (2 strains), *Enterobacter aerogenes, Citrobacter farmeri, Campylobacter jejuni, Serratia marcescens*. Amplification was observed only for the *Escherichia coli* and *Shigella* sp. strains listed and *Escherichia fergusonii*. The sensitivity of the assay with 40-cycle PCR was verified with one strain of *E. coli* and three strains of *Shigella* sp. The detection limit for *E. coli* and *Shigella* sp. was 1 to 10 copies of genomic DNA, depending on the strains tested.

EXAMPLE 29:

Specific detection and identification of *Klebsiella pneumoniae* using *atpD* sequences. The analysis of *atpD* sequences from a variety of bacterial species allowed the selection of PCR primers specific to *K. pneumoniae*. The primer design strategy is similar to the strategy described in Example 28 except that *atpD* sequences were used in the alignment.

Two *K. pneumoniae*-specific primers were selected, (SEQ ID NOs. 1331 and 1332) which give an amplification product of 115 bp. Standard PCR was carried out on PTC-200 thermocyclers (MJ Research) using 0.4 μM of each primer as described in Example 28. The optimal cycling conditions for maximum sensitivity and specificity were as follow: three minutes at 95 °C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 55°C, followed by terminal extension at 72 °C for 2 minutes.

Specificity of the assay was tested by adding to the PCR reactions 0.1 ng of genomic DNA from each of the following bacterial species: *Klebsiella pneumoniae* (2 strains), *Klebsiella ornitholytica, Klebsiella oxytoca* (2 strains), *Klebsiella planticola, Klebsiella terrigena, Citrobacter freundii, Escherichia coli, Salmonella cholerasuis typhi, Serratia marcescens, Enterobacter aerogenes, Proteus vulgaris,*

Kluyvera ascorbata, Kluyvera georgiana, Kluyvera cryocrescens and *Yersinia enterolitica*. Amplification was detected for the two *K. pneumoniae* strains, *K. planticola, K. terrigena* and the three *Kluyvera* species tested. Analysis of the multiple alignment sequence of the *atpD* gene allowed the design of an internal probe SEQ ID NO. 2167 which can discrimate *Klebsiella pneumoniae* from other *Klebsiella* sp. and *Kluyvera* sp. The sensitivity of the assay with 40-cycle PCR was verified with one strain of *K. pneumoniae*. The detection limit for *K. pneumoniae* was around 10 copies of genomic DNA.

EXAMPLE 30:

Specific detection and identification of *Acinetobacter baumannii* using *atpD* sequences. The analysis of *atpD* sequences from a variety of bacterial species allowed the selection of PCR primers specific to *Acinetobacter baumannii*. The primer design strategy is similar to the strategy described in Example 28.

Two *A. baumannii*-specific primers were selected, SEQ ID NOs. 1690 and 1691, which give an amplification product of 233 bp. Standard PCR was carried out on PTC-200 thermocyclers (MJ Research) using 0.4 μM of each primer as described in Example 28. The optimal cycling conditions for maximum sensitivity and specificity were as follow: three minutes at 95 °C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 60°C, followed by terminal extension at 72 °C for 2 minutes.

Specificity of the assay was tested by adding to the PCR reactions 0.1 ng of genomic DNA from each of the following bacterial species: *Acinetobacter baumannii* (3 strains), *Acinetobacter anitratus, Acinetobacter lwoffi, Serratia marcescens, Enterobacter cloacae, Enterococcus faecalis, Pseudomonas aeruginosa, Psychrobacter phenylpyruvicus, Neisseria gonorrheoae, Haemophilus haemoliticus, Yersinia enterolitica, Proteus vulgaris, Eikenella corrodens,*

Escherichia coli. Amplification was detected only for *A. baumannii, A anitratus* and *A. lwoffi*. The sensitivity of the assay with 40-cycle PCR was verified with two strains of *A. baumannii*. The detection limit for the two *A. baumannii* strains tested was 5 copies of genomic DNA. Analysis of the multiple alignment sequence of the *atpD* gene allowed the design of a *A. baumannii*-specific internal probe (SEQ ID NO. 2169).

EXAMPLE 31:

Specific detection and identification of *Neisseria gonorrhoeae* using *tuf* sequences. The analysis of *tuf* sequences from a variety of bacterial species allowed the selection of PCR primers specific to *Neisseria gonorrhoeae*. The primer design strategy is similar to the strategy described in Example 28.

Two *N. gonorrhoeae*-specific primers were selected, SEQ ID NOs. 551 and 552, which give an amplification product of 139 bp. PCR amplification was carried out on PTC-200 thermocyclers (MJ Research) using 0.4 μM of each primer as described in Example 28. The optimal cycling conditions for maximum sensitivity and specificity were as follow: three minutes at 95 °C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 65°C, followed by terminal extension at 72 °C for 2 minutes.

Specificity of the assay was tested by adding into the PCR reactions, 0.1 ng of genomic DNA from each of the following bacterial species: *Neisseria gonorrhoeae* (19 strains), *Neisseria meningitidis* (2 strains), *Neisseria lactamica, Neisseria flavescens, Neisseria animalis, Neisseria canis, Neisseria cuniculi, Neisseria elongata, Neisseria mucosa, Neisseria polysaccharea, Neisseria sicca, Neisseria subflava, Neisseria weaveri*. Amplification was detected only for *N. gonorrhoeae, N. sicca* and *N. polysaccharea*. The sensitivity of the assay with 40-cycle PCR was verified with two strains of *N. gonorrhoeae*. The detection limit for the *N.*

gonorrhoeae strains tested was 5 copies of genomic DNA. Analysis of the multiple alignment sequence of the *tuf* gene allowed the design of an internal probe, SEQ ID NO. 2166, which can discriminate *N. gonorrhoeae* from *N. sicca* and *N. polysaccharea.*

EXAMPLE 32:

Sequencing of bacterial *gyrA* and *parC* gene fragments. Sequencing of bacterial *gyrA* and *parC* fragments. One of the major mechanism of resistance to quinolone in various bacterial species is mediated by target changes (DNA gyrase and/or topoisomerase IV). These enzymes control DNA topology and are vital for chromosome function and replication. Each of these enzymes is a tetramer composed of two subunits: GyrA and GyrB forming A_2B_2 complex in DNA gyrase; and ParC and ParE forming and C_2E_2 complex in DNA topoisomerase IV. It has been shown that they are hotspots, called the quinolone-resitance-determining region (QRDR) for mutations within *gyrA* that encodes for the GyrA subunit of DNA gyrase and within *parC* that encodes the *parC* subunit of topoisomerase IV.

In order to generate a database for *gyrA* and *parC* sequences that can be used for design of primers and/or probes for the specific detection of quinolone resistance in various bacterial species, *gyrA* and *parC* DNA fragments selected from public database (GenBanK and EMBL) from a variety of bacterial species were used to design oligonucleotide primers.

Using primer pair SEQ ID NOs. 1297 and 1298, it was possible to amplify and determine *gyrA* sequences from *Klebsiella oxytoca* (SEQ ID NO. 1764), *Klebsiella pneumoniae* subsp. *ozaneae* (SEQ ID NO. 1765), *Klebsiella planticola* (SEQ ID NO. 1766), *Klebsiella pneumoniae* (SEQ ID NO. 1767), *Klebsiella pneumoniae* subsp. *pneumoniae* (two strains) (SEQ ID NOs. 1768-1769), *Klebsiella*

pneumoniae subsp. *rhinoscleromatis* (SEQ ID NO. 1770), *Klebsiella terrigena* (SEQ ID NO. 1771), *Kluyvera ascorbata* (SEQ ID NO. 2013), *Kluyvera georgiana* (SEQ ID NO. 2014) and *Escherichia coli* (4 strains) (SEQ ID NOs. 2277-2280). Using primer pair SEQ ID NOs. 1291 and 1292, it was possible to amplify and determine *gyrA* sequences from *Legionella pneumophila* subsp. *pneumophila* (SEQ ID NO. 1772), *Proteus mirabilis* (SEQ ID NO. 1773), *Providencia rettgeri* (SEQ ID NO. 1774), *Proteus vulgaris* (SEQ ID NO. 1775) and *Yersinia enterolitica* (SEQ ID NO. 1776). Using primer pair SEQ ID NOs. 1340 and 1341, it was possible to amplify and determine *gyrA* sequence from *Staphylococcus aureus* (SEQ ID NO. 1255).

Using primers SEQ ID NOs. 1318 and 1319, it was possible to amplify and determine *parC* sequences from *K. oxytoca* (two strains) (SEQ ID NOs. 1777-1778), *Klebsiella pneumoniae* subsp. *ozaenae* (SEQ ID NO. 1779), *Klebsiella planticola* (SEQ ID NO. 1780), *Klebsiella pneumoniae* (SEQ ID NO. 1781), *Klebsiella pneumoniae* subsp. *pneumoniae* (two strains) (SEQ ID NOs. 1782-1783), *Klebsiella pneumoniae* subsp. *rhinoscleromatis* (SEQ ID NO. 1784) and *Klebsiella terrigena* (SEQ ID NO. 1785).

EXAMPLE 33:

<u>Development of a PCR assay for the specific detection and identification of *Staphylococcus aureus* and its quinolone resistance genes *gyrA* and *parC*.</u> The analysis of *gyrA* and *parC* sequences from a variety of bacterial species revealed conserved regions allowing the design of PCR primers specific to the quinolone-resistance-determining region (QRDR) of *gyrA* and *parC* from *Staphylococcus aureus*. PCR primer pair SEQ ID NOs. 1340 and 1341 was designed to amplify the *gyrA* sequence of *S. aureus*, whereas PCR primer pair SEQ ID NOs. 1342 and 1343 was designed to amplify *S. aureus parC*. The comparison of *gyrA* and *parC* sequences from *S. aureus* strains with various levels of quinolone resistance

allowed the identification of amino acid substitutions Ser-84 to Leu, Glu-88 to Gly or Lys in the GyrA subunit of DNA gyrase encoded by *gyrA* and amino acid changes Ser-80 to Phe or Tyr and Ala-116 to Glu in the ParC subunit of topoisomerase IV encoded by *parC*. These amino acid substitutions in GyrA and ParC subunits occur in isolates with intermediate- or high-level quinolone resistance. Internal probes for the specific detection of wild-type *S. aureus gyrA* (SEQ ID NO. 1940) and wild-type *S. aureus parC* (SEQ ID NO. 1941) as well as internal probes for the specific detection of each of the *gyrA* (SEQ ID NOs. 1333-1335) and *parC* mutations identified in quinolone-resistant *S. aureus* (SEQ ID NOs. 1336-1339) were designed.

The *gyrA*- and *parC*-specific primer pairs (SEQ ID NOs. 1340-1341 and SEQ ID NOs. 1342-1343) were used in multiplex. PCR amplification was carried out on PTC-200 thermocyclers (MJ Research) using 0.3, 0.3, 0.6 and 0.6 μM of each primers, respectively, as described in Example 28. The optimal cycling conditions for maximum sensitivity and specificity were 3 minutes at 95 °C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 62 °C, followed by terminal extension at 72 °C for 2 minutes. Detection of the PCR products was made by electrophoresis in agarose gels (2 %) containing 0.25 μg/ml of ethidium bromide. The specificity of the multiplex assay with 40-cycle PCR was verified by using 0.1 ng of purified genomic DNA from a panel of gram-positive bacteria. The list included the following: *Abiotrophia adiacens*, *Abiotrophia defectiva*, *Bacillus cereus*, *Bacillus mycoides*, *Enterococcus faecalis* (2 strains), *Enterococcus flavescens*, *Gemella morbillorum*, *Lactococcus lactis*, *Listeria innocua*, *Listeria monocytogenes*, *Staphylococcus aureus* (5 strains), *Staphylococcus auricalis*, *Staphylococcus capitis* subsp. *urealyticus*, *Staphylococcus carnosus*, *Staphylococcus chromogenes*, *Staphylococcus epidermidis* (3 strains), *Staphylococcus gallinarum*, *Staphylococcus haemolyticus* (2 strains), *Staphylococcus hominis*, *Staphylococcus hominis* subsp *hominis*, *Staphylococcuslentus*, *Staphylococcus lugdunensis*, *Staphylococcus*

saccharolyticus, Staphylococcus saprophyticus (3 strains), *Staphylococcus simulans, Staphylococcus warneri, Staphylococcus xylosus, Streptococcus agalactiae, Streptococcus pneumoniae*. Strong amplification of both *gyrA* and *parC* genes was only detected for the *S. aureus* strains tested. The sensitivity of the multiplex assay with 40-cycle PCR was verified with one quinolone-sensitive and four quinolone-resistant strains of *S. aureus*. The detection limit was 2 to 10 copies of genomic DNA, depending on the strains tested.

Detection of the hybridization with the internal probes was performed as described in Example 7. The internal probes specific to wild-type *gyrA* and *parC* of *S. aureus* and to the *gyrA* and *parC* variants of *S. aureus* were able to recognize two quinolone-resistant and one quinolone-sensitive *S. aureus* strains showing a perfect correlation with the susceptibility to quinolones.

The complete assay for the specific detection of *S. aureus* and its susceptibility to quinolone contains the *Staphylococcus*-specific primers (SEQ ID NOs. 553 and 575) described in Example 7 and the multiplex containing the *S. aureus gyrA*- and *parC*-specific primer pairs (SEQ ID NOs. 1340-1341 and SEQ ID NOs. 1342-1343). Amplification is coupled with post-PCR hybridization with the internal probe specific to *S. aureus* (SEQ ID NO. 587) described in Example 7 and the internal probes specific to wild-type *S. aureus gyrA* and *parC* (SEQ ID NOs. 1940-1941) and to the *S. aureus gyrA* and *parC* variants (SEQ ID NOs. 1333-1338).

An assay was also developed for the detection of quinolone-resistant *S. aureus* using the SmartCycler (Cepheid). Real-time detection is based on the use of *S. aureus parC*-specific primers (SEQ ID NOs. 1342 and 1343) and the *Staphylococcus*-specific primers (SEQ ID NOs. 553 and 575) described in Example 7. Internal probes were designed for molecular beacon detection of the wild-type *S. aureus parC* (SEQ ID NO.1939), for detection of the Ser-80 to Tyr or

Phe amino acid substitutions in the ParC subunit encoded by *S. aureus parC* (SEQ ID NOs. 1938 and 1955) and for detection of *S. aureus* (SEQ ID NO. 2282) .

EXAMPLE 34:

Development of a PCR assay for the detection and identification of *Klebsiella pneumoniae* and its quinolone resistance genes *gyrA* and *parC*. The analysis of *gyrA* and *parC* sequences from a variety of bacterial species from the public databases and from the database described in Example 32 revealed conserved regions allowing the design of PCR primers specific to the quinolone-resistance-determining region (QRDR) of *gyrA* and *parC* from *K. pneumoniae*. PCR primer pair SEQ ID NOs. 1936 and 1937, or pair SEQ ID NOs. 1937 and 1942, were designed to amplify the *gyrA* sequence of *K. pneumoniae*, whereas PCR primer pair SEQ ID NOs. 1934 and 1935 was designed to amplify *K. pneumoniae parC* sequence. An alternative pair, SEQ ID NOs. 1935 and 1936, can also amplify *K. pneumoniae parC*. The comparison of *gyrA* and *parC* sequences from *K. pneumoniae* strains with various levels of quinolone resistance allowed the identification of amino acid substitutions Ser-83 to Tyr or Phe and Asp-87 to Gly or Ala and Asp-87 to Asn in the GyrA subunit of DNA gyrase encoded by *gyrA* and amino acid changes Ser-80 to Ile or Arg and Glu-84 to Gly or Lys in the ParC subunit of topoisomerase IV encoded by *parC*. These amino acid substitutions in the GyrA and ParC subunits occur in isolates with intermediate- or high-level quinolone resistance. Internal probes for the specific detection of wild-type *K. pneumoniae gyrA* (SEQ ID NO. 1943) and wild-type *K. pneumoniae parC* (SEQ ID NO. 1944) as well as internal probes for the specific detection of each of the *gyrA* (SEQ ID NOs. 1945-1949) and *parC* mutations identified in quinolone-resistant *K. pneumoniae* (SEQ ID NOs. 1950-1953) were designed.

Two multiplex using the *K. pneumoniae gyrA*- and *parC*-specific primer pairs were used: the first multiplex contained *K. pneumoniae gyrA*-specific primers (SEQ ID

NOs. 1937 and 1942) and *K. pneumoniae parC*-specific primers (SEQ ID NOs. 1934 and 1935) and the second multiplex contained *K. pneumoniae gyrA/parC*-specific primer (SEQ ID NOs. 1936), *K. pneumoniae gyrA*-specific primer (SEQ ID NO. 1937) and *K. pneumoniae parC*-specific primer (SEQ ID NO. 1935). Standard PCR was carried out on PTC-200 thermocyclers (MJ Research) using for the first multiplex 0.6, 0.6, 0.4, 0.4 μM of each primer, respectively, and for the second multiplex 0.8, 0.4, 0.4 μM of each primer, respectively. PCR amplification and agarose gel electrophoresis of the amplified products were performed as described in Example 28. The optimal cycling conditions for maximum sensitivity and specificity were 3 minutes at 95 °C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 62 °C, followed by terminal extension at 72 °C for 2 minutes. The specificity of the two multiplex assays with 40-cycle PCR was verified by using 0.1 ng of purified genomic DNA from a panel of gram-negative bacteria. The list included: *Acinetobacter baumannii*, *Citrobacter freundii*, *Eikenella corrodens*, *Enterobacter aerogenes*, *Enterobacter cancerogenes*, *Enterobacter cloacae*, *Escherichia coli* (10 strains), *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Klebsiella ornitholytica*, *Klebsiella oxytoca* (2 strains), *Klebsiella planticola*, *Klebsiella terrigena*, *Kluyvera ascorbata*, *Kluyvera cryocrescens*, *Kluyvera georgiana*, *Neisseria gonorrhoeae*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella choleraesuis* subsp. *typhimurium*, *Salmonella enteritidis*, *Serratia liquefaciens*, *Serratia marcescens* and *Yersinia enterocolytica*. For both multiplex, strong amplification of both *gyrA* and *parC* was observed only for the *K. pneumoniae* strain tested. The sensitivity of the two multiplex assays with 40-cycle PCR was verified with one quinolone-sensitive strain of *K. pneumoniae*. The detection limit was around 10 copies of genomic DNA.

The complete assay for the specific detection of *K. pneumoniae* and its susceptibility to quinolone contains the *Klebsiella*-specific primers (SEQ ID NOs. 1331 and 1332) described in Example 29 and either the multiplex containing the *K.*

pneumoniae gyrA- and *parC*-specific primers (SEQ ID NOs. 1935, 1936, 1937) or the multiplex containing the *K. pneumoniae gyrA*- and *parC*-specific primers (SEQ ID NOs. 1934, 1937, 1939, 1942). Amplification is coupled with post-PCR hybridization with the internal probe specific to *K. pneumoniae* (SEQ ID NO. 2167) described in Example 29 and the internal probes specific to wild-*type K. pneumoniae gyrA* and *parC* (SEQ ID NOs. 1943, 1944) and to the *K. pneumoniae gyrA* and *parC* variants (SEQ ID NOs. 1945-1949 and 1950-1953).

An assay was also developed for the detection of quinolone-resistant *K. pneumoniae* using the SmartCycler (Cepheid). Real-time detection is based on the use of resistant *K. pneumoniae gyrA*-specific primers (SEQ ID NOs. 1936 and 1937) and the *K. pneumoniae*-specific primers (SEQ ID NOs. 1331 and 1332) described in Example 29. Internal probes were designed for molecular beacon detection of the wild-type *K. pneumoniae gyrA* (SEQ ID NO. 2251), for detection of the Ser-83 to Tyr or Phe and/or Asp-87 to Gly or Asn in the GyrA subunit of DNA gyrase encoded by *gyrA* (SEQ ID NOs. 2250) and for detection of *K. pneumoniae* (SEQ ID NO. 2281).

EXAMPLE 35:

<u>Development of a PCR assay for detection and identification of *S. pneumoniae* and its quinolone resistance genes *gyrA* and *parC*</u>. The analysis of *gyrA* and *parC* sequences from a variety of bacterial species revealed conserved regions allowing the design of PCR primers able to amplify the quinolone-resistance-determining region (QRDR) of *gyrA* and *parC* from all *S. pneumoniae* strains. PCR primer pair SEQ ID NOs. 2040 and 2041 was designed to amplify the QRDR of *S. pneumoniae gyrA*, whereas PCR primer pair SEQ ID NOs. 2044 and 2045 was designed to amplify the QRDR of *S. pneumoniae parC*. The comparison of *gyrA* and *parC* sequences from *S. pneumoniae* strains with various levels of quinolone resistance allowed the identification of amino acid substitutions Ser-81 to Phe or

Tyr in the GyrA subunit of DNA gyrase encoded by *gyrA* and amino acid changes Ser-79 to Phe in the ParC subunit of topoisomerase IV encoded by *parC*. These amino acid substitutions in the GyrA and ParC subunits occur in isolates with intermediate- or high-level quinolone resistance. Internal probes for the specific detection of each of the *gyrA* (SEQ ID NOs. 2042 and 2043) and *parC* (SEQ ID NO. 2046) mutations identified in quinolone-resistant *S. pneumoniae* were designed.

For all bacterial species, amplification was performed from purified genomic DNA. 1 μl of genomic DNA at 0.1 ng/μL was transferred directly to a 19 μl PCR mixture. Each PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM $MgCl_2$, 0.4 μM (each) of the above primers SEQ ID NOs. 2040, 2041, 2044 and 2045, 0.05 mM bovine serum albumin (BSA) and 0.5 U *Taq* polymerase coupled with TaqStart™ antibody. The optimal cycling conditions for maximum sensitivity and specificity were 3 minutes at 95 °C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 58 °C, followed by terminal extension at 72 °C for 2 minutes. In order to generate Digoxigenin (DIG)-labeled amplicons for capture probe hybridization, 0.1X PCR DIG labeling four deoxynucleoside triphosphates mix (Boehringer Mannheim GmbH) was used for amplification.

The DIG–labeled amplicons were hybridized to the capture probes bound to 96-well plates. The plates were incubated with anti-DIG-alkaline phosphatase and the chemiluminescence was measured by using a luminometer (MLX, Dynex Technologies Inc.) after incubation with CSPD and recorded as Relative Light Unit (RLU). The RLU ratio of tested sample with and without captures probes was then calculated. A ratio $\geq$ 2.0 was defined as a positive hybridization signal. All reactions were performed in duplicate.

The specificity of the multiplex assay with 40-cycle PCR was verified by using 0.1 ng of purified genomic DNA from a panel of bacteria listed in Table 13. Strong amplification of both *gyrA* and *parC* was detected only for the *S. pneumoniae* strains tested. Weak amplification of both *gyrA* and *parC* genes was detected for *Staphylococcus simulans*. The detection limit tested with purified genomic DNA from 5 strains of *S. pneumoniae* was 1 to 10 genome copies. In addition, 5 quinolone-resistant and 2 quinolone-sensitive clinical isolates of *S. pneumoniae* were tested to further validate the developed multiplex PCR coupled with capture probe hybridization assays. There was a perfect correlation between detection of *S. pneumoniae gyrA* and *parC* mutations and the susceptibility to quinolone.

The complete assay for the specific detection of *S. pneumoniae* and its susceptibility to quinolone contains the *S. pneumoniae*-specific primers (SEQ ID NOs. 1179 and 1181) described in Exemple 20 and the multiplex containing the *S. pneumoniae gyrA*-specific and *parC*-specific primer pairs (SEQ ID NOS. 2040 and 2041 and SEQ ID NOs. 2044 and 2045). Amplification is coupled with post-PCR hybridization with the internal probe specific to *S. pneumoniae* (SEQ ID NO. 1180) described in Example and the internal probes specific to each of the *S. pneumoniae gyrA* and *parC* variants (SEQ ID NOs. 2042, 2043 and 2046).

EXAMPLE 36:

Detection of extended-spectrum TEM-type β-lactamases in *Escherichia coli*. The analysis of TEM sequences which confer resistance to third-generation cephalosporins and to β-lactamase inhibitors allowed the identification of amino acid substitutions Met-69 to Ile or Leu or Val, Ser-130 to Gly, Arg-164 to Ser or His, Gly-238 to Ser , Glu-240 to Lys and Arg-244 to Ser or Cys or Thr or His or Leu. PCR primers SEQ ID NOs. 1907 and 1908 were designed to amplify TEM sequences. Internal probes for the specific detection of wild-type TEM (SEQ ID NO. 2141) and for each of the amino acid substitutions (SEQ ID NOs. 1909-1926) identified in TEM variants were designed to detect resistance to third-generation

cephalosporins and to β-lactamase inhibitors. Design and synthesis of primers and probes, and detection of the hybridization were performed as described in Example 7.

For all bacterial species, amplification was performed from purified genomic DNA. One μl of genomic DNA at 0.1ng/μl was transferred directly to a 19 μl PCR mixture. Each PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0); 0.1% Triton X-100, 2.5 mM $MgCl_2$, 0.4 μM of the TEM-specific primers SEQ ID NOs. 1907 and 1908, 200 μM (each) of the four deoxynucleoside triphosphates, 0.05 mM bovine serum albumin (BSA) and 0.5 U *Taq* polymerase (Promega) coupled with TaqStart™ antibody. PCR amplification and agarose gel analysis of the amplified products were performed as described in Example 28. The optimal cycling conditions for maximum sensitivity and specificity were 3 minutes at 95 °C for initial denaturation, then forty cycles of three steps consisting of 5 seconds at 95 °C, 30 seconds at 55 °C and 30 seconds at 72 °C, followed by terminal extension at 72 °C for 2 minutes.

The specificity of the TEM-specific primers with 40-cycle PCR was verified by using 0.1 ng of purified genomic from the following bacteria: three third-generation cephalosporin-resistant *Escherichia coli* strains (one with TEM-10, one with TEM-28 and the other with TEM-49), two third-generation cephalosporin-sensitive *Escherichia coli* strain (one with TEM-1 and the other without TEM), one third-generation cephalosporin-resistant *Klebsiella pneumoniae* strain (with TEM-47), and one β-lactamase-inhibitor-resistant *Proteus mirabilis* strain (with TEM-39). Amplification with the TEM-specific primers was detected only for strains containing TEM.

The sensitivity of the assay with 40-cycle PCR was verified with three *E. coli* strains containing TEM-1 or TEM-10 or TEM-49, one *K. pneumoniae* strain containing TEM-47 and one *P. mirabilis* strain containing TEM-39. The detection

limit was 5 to 100 copies of genomic DNA, depending on the TEM-containing strains tested.

The TEM-specific primers SEQ ID NOs. 1907 and 1908 were used in multiplex with the *Escherichia coli/Shigella sp.*–specific primers SEQ ID NOs. 1661 and 1665 described in Example 28 to allow the complete identification of *Escherichia coli/Shigella sp.* and the susceptibility to β-lactams. PCR amplification with 0.4 μM of each of the primers and agarose gel analysis of the amplified products was performed as described above.

The specificity of the multiplex with 40-cycle PCR was verified by using 0.1 ng of purified genomic DNA from the following bacteria: three third-generation cephalosporin-resistant *Escherichia coli* strains (one with TEM-10, one with TEM-28 and the other with TEM-49), two third-generation cephalosporin-sensitive *Escherichia coli* strain (one with TEM-1 and the other without TEM), one third-generation cephalosporin-resistant *Klebsiella pneumoniae* strain (with TEM-47), and one β-lactamase-inhibitor-resistant *Proteus mirabilis* strain (with TEM-39). The multiplex was highly specific to *Escherichia coli* strains containing TEM.

The complete assay for detection of TEM-type β-lactamases in *E. coli* includes PCR amplification using the multiplex containing the TEM-specific primers (SEQ ID NOs. 1907 and 1908) and the *Escherichia coli/Shigella* sp.-specific primers (SEQ ID NOs. 1661 and 1665) coupled with post PCR-hybridization with the internal probes specific to wild-type TEM (SEQ ID NO. 2141) and to the TEM variants (SEQ ID NOs. 1909-1926).

EXAMPLE 37:

Detection of extended-spectrum SHV-type β-lactamases in *Klebsiella pneumoniae*.

The comparison of SHV sequences, which confer resistance to third-generation

cephalosporins and to β-lactamase inhibitors, allowed the identification of amino acid substitutions Ser-130 to Gly, Asp-179 to Ala or Asn, Gly-238 to Ser , and Glu-240 to Lys. PCR primer pair SEQ ID NOs. 1884 and 1885 was designed to amplify SHV sequences. Internal probes for the specific identification of wild-type SHV (SEQ ID NO. 1896) and for each of the amino acid substitutions (SEQ ID NOs. 1886-1895 and 1897-1898) identified in SHV variants were designed to detect resistance to third-generation cephalosporins and to β-lactamase inhibitors. Design and synthesis of primers and probes, and detection of the hybridization were performed as described in Example 7.

For all bacterial species, amplification was performed from purified genomic DNA. One μl of of genomic DNA at 0.1ng/μl was transferred directly to a 19 μl PCR mixture. Each PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM $MgCl_2$, 0.4 μM of the SHV-specific primers SEQ ID NO. 1884 and 1885, 200 μM (each) of the four deoxynucleoside triphosphates, 0.05 mM bovine serum albumin (BSA) and 0.5 U *Taq* polymerase (Promega) coupled with TaqStart™ antibody. PCR amplification and agarose gel analysis of the amplified products were performed as described in Example 28. The optimal cycling conditions for maximum sensitivity and specificity were 3 minutes at 95 °C for initial denaturation, then forty cycles of three steps consisting of 5 seconds at 95 °C, 30 seconds at 55 °C and 30 seconds at 72 °C, followed by terminal extension at 72 °C for 2 minutes.

The specificity of the SHV-specific primers with 40-cycle PCR was verified by using 0.1 ng of purified genomic from the following bacteria: two third-generation cephalosporin-resistant *Klebsiella pneumoniae* strains (one with SHV-2a and the other with SHV-12), one third-generation cephalosporin-sensitive *Klebsiella pneumoniae* strain (with SHV-1), two third-generation cephalosporin-resistant *Escherichia coli* strains (one with SHV-8 and the other with SHV-7), and two third-generation cephalosporin-sensitive *Escherichia coli* strains (one with SHV-1

and the other without any SHV). Amplification with the SHV-specific primers was detected only for strains containing SHV.

The sensitivity of the assay with 40-cycle PCR was verified with four strains containing SHV. The detection limit was 10 to 100 copies of genomic DNA, depending on the SHV-containing strains tested.

The amplification was coupled with post-PCR hybridization with the internal probes specific for identification of wild-type SHV (SEQ ID NO. 1896) and for each of the amino acid substitutions (SEQ ID NOs. 1886-1895 and 1897-1898) identified in SHV variants. The specificity of the probes was verified with six strains containing various SHV enzymes, one *Klebsiella pneumoniae* strain containing SHV-1, one *Klebsiella pneumoniae* strain containing SHV-2a, one *Klebsiella pneumoniae* strain containing SHV-12, one *Escherichia coli* strain containing SHV-1, one *Escherichia coli* strain containing SHV-7 and one *Escherichia coli* strain containing SHV-8. The probes correctly detected each of the SHV genes and their specific mutations. There was a perfect correlation between the SHV genotype of the strains and the susceptibility to β-lactam antibiotics.

The SHV-specific primers SEQ ID NOs. 1884 and 1885 were used in multiplex with the *K. pneumoniae*–specific primers SEQ ID NOs. 1331 and 1332 described in Example 29 to allow the complete identification of *K. pneumoniae* and the susceptibility to β-lactams. PCR amplification with 0.4 μM of each of the primers and agarose gel analysis of the amplified products were performed as described above.

The specificity of the multiplex with 40-cycle PCR was verified by using 0.1 ng of purified genomic DNA from the following bacteria: three *K. pneumoniae* strains containing SHV-1, one *Klebsiella pneumoniae* strain containing SHV-2a, one

Klebsiella pneumoniae strain containing SHV-12, one *K. rhinoscleromatis* strain containing SHV-1, one *Escherichia coli* strain without SHV. The multiplex was highly specific to *Klebsiella pneumoniae* strain containing SHV.

EXAMPLE 38:

Development of a PCR assay for the detection and identification of *Neisseria gonorrhoeae* and its associated tetracycline resistance gene *tetM*. The analysis of publicly available *tetM* sequences revealed conserved regions allowing the design of PCR primers specific to *tetM* sequences. The PCR primer pair SEQ ID NOs. 1588 and 1589 was used in multiplex with the *Neisseria gonorrhoeae*-specific primers SEQ ID NOs. 551 and 552 described in Example 31. Sequence alignment analysis of *tetM* sequences revealed regions suitable for the design of an internal probe specific to *tetM* (SEQ ID NO. 2254). PCR amplification was carried out on PTC-200 thermocyclers (MJ Research) using 0.4 μM of each primer pair as described in Example 28. The optimal cycling conditions for maximum sensitivity and specificity were as follow: three minutes at 95 °C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 60°C, followed by terminal extension at 72 °C for 2 minutes.

The specificity of the multiplex PCR assay with 40-cycle PCR was verified by using 0.1 ng of purified genomic DNA from the following bacteria: two tetracycline-resistant *Escherichia coli* strains (one containing the tetracycline-resistant gene *tetB* and the other containing the tetracycline-resistant gene *tetC*), one tetracycline-resistant *Pseudomonas aeruginosa* strain (containing the tetracycline-resistant gene *tetA*), nine tetracycline-resistant *Neisseria gonorrhoeae* strains, two tetracycline-sensitive *Neisseria meningitidis* strains, one tetracycline-sensitive *Neisseria polysaccharea* strain, one tetracycline-sensitive *Neisseria sicca* strain and one tetracycline-sensitive *Neisseria subflava* strain. Amplification with both the *tetM*-specific and *Neisseria gonorrhoeae*-specific primers was detected

only for *N. gonorrhoeae* strains containing *tetM*. There was a weak amplification signal using *Neisseria gonorrhoeae*-specific primers for the following species: *Neisseria sicca, Neisseria polysaccharea* and *Neisseria meningitidis*. There was a perfect correlation between the *tetM* genotype and the tetracycline susceptibility pattern of the *Neisseria gonorrhoeae* strains tested. The internal probe specific to *N. gonorrhoeae* SEQ ID NO. 2166 described in Example 31 can discriminate *Neisseria gonorrhoeae* from the other *Neisseria* sp.

The sensitivity of the assay with 40-cycle PCR was verified with two tetracycline resistant strains of *N. gonorrhoeae*. The detection limit was 5 copies of genomic DNA for both strains.

EXAMPLE 39:

Development of a PCR assay for the detection and identification of *Shigella* sp. and their associated trimethoprim resistance gene *dhfrIa*. The analysis of publicly available *dhfrIa* and other *dhfr* sequences revealed regions allowing the design of PCR primers specific to *dhfrIa* sequences. The PCR primer pair (SEQ ID NOs. 1459 and 1460) was used in multiplex with the *Escherichia coli/Shigella* sp.-specific primers SEQ ID NOs. 1661 and 1665 described in Example 28. Sequence alignment analysis of *dhfrIa* sequences revealed regions suitable for the design of an internal probe specific to *dhfrIa* (SEQ ID NO. 2253). PCR amplification and agarose gel analysis of the amplified products were performed as described in Example 28 with an annealing temperature of 60 °C. The specificity of the multiplex assay with 40-cycle PCR was verified by using 0.1 ng of purified genomic DNA from a panel of bacteria. The list included the following trimethoprim-sensitive strains, *Salmonella typhimyurium, Salmonella typhi, Salmonella enteritidis, Tatumella ptyseos, Klebsiella pneumoniae, Enterobacter aerogenes, Citrobacter farmeri, Campylobacter jejuni, Serratia marcescens, Shigella dysenteriae, Shigella flexneri, Shigella sonnei*, six trimethoprim-resistant *Escherichia coli* strains (containing *dhfrIa* or *dhfrV* or *dhfrVII* or *dhfrXII* or

dhfrXIII or *dhfrXV*), four trimethoprim-resistant strains containing *dhfrIa* (*Shigella sonnei, Shigella flexneri, Shigella dysenteriae* and *Escherichia coli*). There was a perfect correlation between the *dhfrIa* genotype and the trimethoprim susceptibility pattern of the *Escherichia coli* and *Shigella* sp. strains tested. The *dhfrIa* primers were specific to the *dhfrIa* gene and did not amplify any of the other trimethoprim-resistant *dhfr* genes tested. The sensitivity of the multiplex assay with 40-cycle PCR was verified with three strains of trimethoprim-resistant strains of *Shigella* sp. The detection limit was 5 to 10 genome copies of DNA, depending on the *Shigella* sp. strains tested.

EXAMPLE 40:

<u>Development of a PCR assay for the detection and identification of *Acinetobacter baumannii* and its associated aminoglycoside resistance gene *aph(3')-VIa*.</u> The comparison of publicly available *aph(3')-VIa* sequence revealed regions allowing the design of PCR primers specific to *aph(3')-VIa*. The PCR primer pair (SEQ ID NOs. 1404 and 1405) was used in multiplex with the *Acinetobacter baumannii*-specific primers SEQ ID NOs. 1692 and 1693 described in Example 30. Analysis of the *aph(3')-VIa* sequence revealed region suitable for the design of an internal probe specific to *aph(3')-VIa* (SEQ ID NO. 2252). PCR amplification and agarose gel analysis of the amplified products were performed as described in Example 28. The specificity of the multiplex assay with 40-cycle PCR was verified by using 0.1 ng of purified genomic DNA from a panel of bacteria including: two aminoglycoside-resistant *A. baumanni* strains (containing *aph(3')-VIa*), one aminoglycoside-sensitive *A. baumani* strain, one of each of the following aminoglycoside-resistant bacteria, one *Serratia marcescens* strain containing the aminoglycoside-resistant gene *aacC1*, one *Serratia marcescens* strain containing the aminoglycoside-resistant gene *aacC4*, one *Enterobacter cloacae* strain containing the aminoglycoside-resistant gene *aacC2*, one *Enterococcus faecalis* containing the aminoglycoside-resistant gene *aacA-aphD*, one *Pseudomonas*

aeruginosa strain containing the aminoglycoside-resistant gene *aac6IIa* and one of each of the following aminoglycoside-sensitive bacterial species, *Acinetobacter anitratus, Acinetobacter lwoffi, Psychobbacter phenylpyruvian, Neisseria gonorrhoeae, Haemophilus haemolyticus, Haemophilus influenzae, Yersinia enterolitica, Proteus vulgaris, Eikenella corrodens, Escherichia coli*. There was a perfect correlation between the *aph(3')-VIa* genotype and the aminoglycoside susuceptibility pattern of the *A. baumannii* strains tested. The *aph(3')-VIa*-specific primers were specific to the *aph(3')-VIa* gene and did not amplify any of the other aminoglycoside-resistant genes tested. The sensitivity of the multiplex assay with 40-cycle PCR was verified with two strains of aminoglycoside-resistant strains of *A. baumannii*. The detection limit was 5 genome copies of DNA for both *A. baumannii* strains tested.

EXAMPLE 41:

Specific identification of *Bacteroides fragilis* using *atpD* (V-type) sequences.The comparison of *atpD* (V-type) sequences from a variety of bacterial species allowed the selection of PCR primers for *Bacteroides fragilis*. The strategy used to design the PCR primers was based on the analysis of a multiple sequence alignement of various *atpD* sequences from *B. fragilis*, as well as a*tpD* sequences from the related species *B. dispar*, bacterial genera and archaea, especially representatives with phylogenetically related *atpD* sequences. A careful analysis of this alignment allowed the selection of oligonucleotide sequences which are conserved within the target species but which discriminate sequences from other species, especially from closely related species *B. dispar*, thereby permitting the species-specific and ubiquitous detection and identification of the target bacterial species.

The chosen primer pair, SEQ ID NOs. 2134-2135, produces an amplification product of 231 bp. Standard PCR was carried out on PTC-200 thermocyclers (MJ Research Inc.) using 0.4μM of each primers pair as described in Example 28. The

optimal cycling conditions for maximum sensitivity and specificity were as follows: three minutes at 95°C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95°C and 30 seconds at 60°C, followed by terminal extension at 72°C for 2 minutes.

The format of this assay is not limited to the one described above. A person skilled in the art could adapt the assay for different formats such as PCR with real-time detection using molecular beacon probes. Molecular beacon probes designed to be used in this assay include, but are not limited to, SEQ ID NO. 2136 for the detection of the *B. fragilis* amplicon.

EXAMPLE 42:

Evidence for horizontal gene transfer in the evolution of the elongation factor Tu in Enterococci.

ABSTRACT

The elongation factor Tu, encoded by *tuf* genes, is a GTP binding protein that plays a central role in protein synthesis. One to three *tuf* genes per genome are present depending on the bacterial species. Most low G+C gram-positive bacteria carry only one *tuf* gene. We have designed degenerate PCR primers derived from consensus sequences of the *tuf* gene to amplify partial *tuf* sequences from 17 enterococcal species and other phylogenetically related species. The amplified DNA fragments were sequenced either by direct sequencing or by sequencing cloned inserts containing putative amplicons. Two different *tuf* genes (*tufA* and *tufB*) were found in 11 enterococcal species, including *Enterococcus avium*, *E. casseliflavus*, *E. dispar*, *E. durans*, *E. faecium*, *E. gallinarum*, *E. hirae*, *E. malodoratus*, *E. mundtii*, *E. pseudoavium*, and *E. raffinosus*. For the other six enterococcal species (*E. cecorum*, *E. columbae*, *E. faecalis*, *E. sulfureus*, *E.*

saccharolyticus, and *E. solitarius*), only the *tufA* gene was present. Based on 16S rRNA gene sequence analysis, the 11 species having two *tuf* genes all share a common ancestor, while the six species having only one copy diverged from the enterococcal lineage before that common ancestor. The presence of one or two copies of the *tuf* gene in enterococci was confirmed by Southern hybridization. Phylogenetic analysis of *tuf* sequences demonstrated that the enterococcal *tufA* gene branches with the *Bacillus*, *Listeria* and *Staphylococcus* genera, while the enterococcal *tufB* gene clusters with the genera *Streptococcus* and *Lactococcus*. Primary structure analysis showed that four amino acid residues within the sequenced regions are conserved and unique to the enterococcal *tufB* genes and the *tuf* genes of streptococci and *L. lactis*. The data suggest that an ancestral streptococcus or a streptococcus-related species may have horizontally transferred a *tuf* gene to the common ancestor of the 11 enterococcal species which now carry two *tuf* genes.

INTRODUCTION

The elongation factor Tu (EF-Tu) is a GTP binding protein playing a central role in protein synthesis. It mediates the recognition and transport of aminoacyl-tRNAs and their positioning to the A-site of the ribosome. The highly conserved function and ubiquitous distribution render the elongation factor a valuable phylogenetic marker among eubacteria and even throughout the archaebacterial and eukaryotic kingdoms. The *tuf* genes encoding elongation factor Tu are present in various copy numbers per bacterial genome. Most gram-negative bacteria contain two *tuf* genes. As found in *Escherichia coli,* the two genes, while being almost identical in sequence, are located in different parts of the bacterial chromosome. However, recently completed microbial genomes revealed that only one *tuf* gene is found in *Helicobacter pylori* as well as in some obligate parasitic bacteria, such as *Borrelia burgdorferi, Rickettsia prowazekii,* and *Treponema pallidum*, and in some cyanobacteria. In most gram-positive bacteria studied so far, only one *tuf* gene was found. However, Southern hybridization showed that there are two *tuf* genes in

some clostridia as well as in *Streptomyces coelicolor* and *S. lividans*. Up to three *tuf*-like genes have been identified in *S. ramocissimus*.

Although massive prokaryotic gene transfer is suggested to be one of the factors responsible for the evolution of bacterial genomes, the genes encoding components of the translation machinery are thought to be highly conserved and difficult to be transferred horizontally due to the complexity of their interactions. However, a few recent studies demonstrated evidence that horizontal gene transfer has also occurred in the evolution of some genes coding for the translation apparatus, namely, 16S rRNA and some aminoacyl-tRNA synthetases. No further data suggest that such a mechanism is involved in the evolution of the elongation factors. Previous studies concluded that the two copies of *tuf* genes in the genomes of some bacteria resulted from an ancient event of gene duplication. Moreover, a study of the *tuf* gene in *R. prowazekii* suggested that intrachromosomal recombination has taken place in the evolution of the genome of this organism.

To date, little is known about the *tuf* genes of enterococcal species. In this study, we analyzed partial sequences of *tuf* genes in 17 enterococcal species, namely, *E. avium*, *E. casseliflavus*, *E. cecorum*, *E. columbae*, *E. dispar*, *E. durans*, *E. faecalis*, *E. faecium*, *E. gallinarum*, *E. hirae*, *E. malodoratus*, *E. mundtii*, *E. pseudoavium*, *E. raffinosus*, *E. saccharolyticus*, *E. solitarius*, and *E. sulfureus*. We report here the presence of two divergent copies of *tuf* genes in 11 of these enterococcal species. The 6 other species carried a single *tuf* gene. The evolutionary implications are discussed.

MATERIALS AND METHODS

Bacterial strains. Seventeen enterococcal strains and other gram-positive bacterial strains obtained from the American Type Culture Collection (ATCC, Manassas, Va.) were used in this study (Table 16). All strains were grown on sheep blood agar or in brain-heart infusion broth prior to DNA isolation.

DNA isolation. Bacterial DNAs were prepared using the G NOME DNA extraction kit (Bio101, Vista, Calif.) as previously described.

Sequencing of putative *tuf* genes. In order to obtain the *tuf* gene sequences of enterococci and other gram-positive bacteria, two sequencing approaches were used: 1) sequencing of cloned PCR products and 2) direct sequencing of PCR products. A pair of degenerate primers (SEQ ID NOs. 664 and 697) were used to amplify an 886-bp portion of the *tuf* genes from enterococcal species and other gram-positive bacteria as previously described. For *E. avium*, *E. casseliflavus*, *E. dispar*, *E. durans*, *E. faecium*, *E. gallinarum*, *E. hirae*, *E. mundtii*, *E. pseudoavium*, and *E. raffinosus*, the amplicons were cloned using the Original TA cloning kit (Invitrogen, Carlsbad, Calif.) as previously described. Five clones for each species were selected for sequencing. For *E. cecorum*, *E. faecalis*, *E. saccharolyticus*, and *E. solitarius* as well as the other gram-positive bacteria, the sequences of the 886-bp amplicons were obtained by direct sequencing. Based on the results obtained from the earlier rounds of sequencing, two pairs of primers were designed for obtaining the partial *tuf* sequences from the other enterococcal species by direct sequencing. One pair of primers (SEQ ID NOs. 543 and 660) were used to amplify the enterococcal *tuf* gene fragments from *E. columbae*, *E. malodoratus*, and *E. sulfureus*. Another pair of primers (SEQ ID NOs. 664 and 661) were used to amplify the second *tuf* gene fragments from *E. avium*, *E. malodoratus*, and *E. pseudoavium*.

Prior to direct sequencing, PCR products were electrophoresed on 1% agarose gel at 120V for 2 hours. The gel was then stained with 0.02% methylene blue for 30 minutes and washed twice with autoclaved distilled water for 15 minutes. The gel slices containing PCR products of the expected sizes were cut out and purified with the QIAquick gel extraction kit (QIAgen Inc., Mississauga, Ontario, Canada) according to the manufacturer's instructions. PCR mixtures for sequencing were prepared as described previously. DNA sequencing was carried out with the Big Dye™ Terminator Ready Reaction cycle sequencing kit using a 377 DNA sequencer (PE Applied Biosystems, Foster City, Calif.). Both strands of the

amplified DNA were sequenced. The sequence data were verified using the Sequencer™ 3.0 software (Gene Codes Corp., Ann Arbor, Mich.).

Sequence analysis and phylogenetic study. Nucleotide sequences of the *tuf* genes and their respective flanking regions for *E. faecalis*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*, were retrieved from the TIGR microbial genome database and *S. pyogenes* from the University of Oklahoma database. DNA sequences and deduced protein sequences obtained in this study were compared with those in all publicly available databases using the BLAST and FASTA programs. Unless specified, sequence analysis was conducted with the programs from GCG package (Version 10; Genetics Computer Group, Madison, Wisc.). Sequence alignment of the *tuf* genes from 74 species representing all three kingdoms of life (Tables 16 and 17) were carried out by use of Pileup and corrected upon visual analysis. The N- and C-termini extremities of the sequences were trimmed to yield a common block of 201 amino acids sequences and equivocal residues were removed. Phylogenetic analysis was performed with the aid of PAUP 4.0b4 written by Dr. David L. Swofford (Sinauer Associates, Inc., Publishers, Sunderland, Mass.). The distance matrix and maximum parsimony were used to generate phylogenetic trees and bootstrap resampling procedures were performed using 500 and 100 replications in each analysis, respectively.

Protein structure analysis. The crystal structures of (i)*Thermus aquaticus* EF-Tu in complex with Phe-tRNA^{Phe} and a GTP analog and (ii) *E. coli* EF-Tu in complex with GDP served as templates for constructing the equivalent models for enterococcal EF-Tu. Homology modeling of protein structure was performed using the SWISS-MODEL server and inspected using the SWISS-PDB viewer version 3.1.

Southern hybridization. In a previous study, we amplified and cloned an 803-bp PCR product of the *tuf* gene fragment from *E. faecium.* Two divergent sequences of the inserts, which we assumed to be *tufA* and *tufB* genes, were obtained. The recombinant plasmid carrying either *tufA* or *tufB* sequence was used to generate two probes labeled with Digoxigenin (DIG)-11-dUTP by PCR

incorporation following the instructions of the manufacturer (Boehringer Mannheim, Laval, Québec, Canada). Enterococcal genomic DNA samples (1-2 µg) were digested to completion with restriction endonucleases *Bgl*II and *Xba*I as recommended by the supplier (Amersham Pharmacia Biotech, Mississauga, Ontario, Canada). These restriction enzymes were chosen because no restriction sites were observed within the amplified *tuf* gene fragments of most enterococci. Southern blotting and filter hybridization were performed using positively charged nylon membranes (Boehringer Mannheim) and QuikHyb hybridization solution (Stratagene Cloning Systems, La Jolla, Calif.) according to the manufacturers' instructions with modifications. Twenty µl of each digestion were electrophoresed for 2 h at 120V on a 0.8% agarose gel. The DNA fragments were denatured with 0.5 M NaOH and transferred by Southern blotting onto a positively charged nylon membrane (Boehringer Mannheim). The filters were pre-hybridized for 15 min and then hybridized for 2 h in the QuikHyb solution at 68°C with either DIG-labeled probe. Posthybridization washings were performed twice with 0.5x SSC, 1% SDS at room temperature for 15 min and twice in the same solution at 60°C for 15 min. Detection of bound probes was achieved using disodium 3- (4-methoxyspiro (1,2-dioxetane-3,2'- (5'-chloro) tricyclo(3,3.1.$1^{3.7}$) decan)-4-yl) phenyl phosphate (CSPD) (Boehringer Mannheim) as specified by the manufacturer.

GenBank submission. The GenBank accession numbers for partial *tuf* gene sequences generated in this study are given in Table 16.

RESULTS

Sequencing and nucleotide sequence analysis. In this study, all gram-positive bacteria other than enterococci yielded a single *tuf* sequence of 886 bp using primers SEQ ID NOs. 664 and 697 (Table 16). Each of four enterococcal species including *E. cecorum*, *E. faecalis*, *E. saccharolyticus*, and *E. solitarius* also yielded one 886-bp *tuf* sequence. On the other hand, for *E. avium*, *E. casseliflavus*, *E. dispar*, *E. durans*, *E. faecium*, *E. gallinarum*, *E. hirae*, *E. mundtii*, *E. pseudoavium*,

and *E. raffinosus*, direct sequencing of the 886-bp fragments revealed overlapping peaks according to their sequence chromatograms, suggesting the presence of additional copies of the *tuf* gene. Therefore, the *tuf* gene fragments of these 10 species were cloned first and then sequenced. Sequencing data revealed that two different types of *tuf* sequences (*tufA* and *tufB*) are found in eight of these species including *E. casseliflavus*, *E. dispar*, *E. durans*, *E. faecium*, *E. gallinarum*, *E. hirae*, *E. mundtii*, and *E. raffinosus*. Five clones from *E. avium* and *E. pseudoavium* yielded only a single *tuf* sequence. These new sequence data allowed the design of new primers specific for the enterococcal *tufA* or *tufB* sequences. Primers SEQ ID NOs. 543 and 660 were designed to amplify only enterococcal *tufA* sequences and a 694-bp fragment was amplified from all 17 enterococcal species. The 694-bp sequences of *tufA* genes from *E. columbae*, *E. malodoratus*, and *E. sulfureus* were obtained by direct sequencing using these primers. Primers SEQ ID NOs. 664 and 661 were designed for the amplification of 730-bp portion of *tufB* genes and yielded the expected fragments from 11 enterococcal species, including *E. malodoratus* and the 10 enterococcal species in which heterogeneous *tuf* sequences were initially found. The sequences of the *tufB* fragments for *E. avium*, *E. malodoratus* and *E. pseudoavium* were determined by direct sequencing using the primers SEQ ID NOs. 664 and 661. Overall, *tufA* gene fragments were obtained from all 17 enterococcal species but *tufB* gene fragments were obtained with only 11 enterococcal species (Table 16).

The identities between *tufA* and *tufB* for each enterococcal species were 68-79% at the nucleotide level and 81 to 89% at the amino acid level. The *tufA* gene is highly conserved among all enterococcal species with identities varying from 87% to 99% for DNA and 93% to 99% for amino acid sequences, while the identities among *tufB* genes of enterococci varies from 77% to 92% for DNA and 91% to 99% for amino acid sequences, indicating their different origins and evolution (Table 18). Since *E. solitarius* has been transferred to the genus *Tetragenococcus*, which is also a low G+C gram-positive bacterium, our sequence comparison did not include this species as an enterococcus. G+C content of enterococcal *tufA*

sequences ranged from 40.8% to 43.1%, while that of enterococcal *tufB* sequences varied from 37.8% to 46.3%. Based on amino acid sequence comparison, the enterococcal *tufA* gene products share higher identities with those of *Abiotrophia adiacens*, *Bacillus subtilis*, *Listeria monocytogenes*, *S. aureus*, and *S. epidermidis*. On the other hand, the enterococcal *tufB* gene products share higher percentages of amino acid identity with the *tuf* genes of *S. pneumoniae*, *S. pyogenes* and *Lactococcus lactis* (Table 18).

In order to elucidate whether the two enterococcal *tuf* sequences encode genuine EF-Tu, the deduced amino acid sequences of both genes were aligned with other EF-Tu sequences available in SWISSPROT (Release 38). Sequence alignment demonstrated that both gene products are highly conserved and carry all conserved residues present in this portion of prokaryotic EF-Tu (Figure 4). Therefore, it appears that both gene products could fulfill the function of EF-Tu. The partial *tuf* gene sequences encode the portion of EF-Tu from residues 117 to 317, numbered as in *E. coli*. This portion makes up of the last four α-helices and two β-strands of domain I, the entire domain II and the N-terminal part of domain III on the basis of the determined structures of *E. coli* EF-Tu.

Based on the deduced amino acid sequences, the enterococcal *tufB* genes have unique conserved residues Lys129, Leu140, Ser230, and Asp234 (*E. coli* numbering) that are also conserved in streptococci and *L. lactis*, but not in the other bacteria (Figure 4). All these residues are located in loops except for Ser230. In other bacteria the residue Ser230 is substituted for highly conserved Thr, which is the 5th residue of the third β-strand of domain II. This region is partially responsible for the interaction between the EF-Tu and aminoacyl-tRNA by the formation of a deep pocket for any of the 20 naturally occurring amino acids. According to our three-dimensional model (data not illustrated), the substitution Thr230→Ser in domain II of EF-Tu may have little impact on the capability of the pocket to accommodate any amino acid. However, the high conservation of Thr230 comparing to the unique Ser substitution found only in streptococci and 11 enterococci could suggest a subtle functional role for this residue.

The *tuf* gene sequences obtained for *E. faecalis*, *S. aureus*, *S. pneumoniae* and *S. pyogenes* were compared with their respective incomplete genome sequence. Contigs with more than 99% identity were identified. Analysis of the *E. faecalis* genome data revealed that the single *E. faecalis tuf* gene is located within an *str* operon where *tuf* is preceded by *fus* that encodes the elongation factor G. This *str* operon is present in *S. aureus* and *B. subtilis* but not in the two streptococcal genomes examined. The 700-bp or so sequence upstream the *S. pneumoniae tuf* gene has no homology with any known gene sequences. In *S. pyogenes*, the gene upstream of *tuf* is similar to a cell division gene, *ftsW*, suggesting that the *tuf* genes in streptococci are not arranged in a *str* operon.

Phylogenetic analysis. Phylogenetic analysis of the *tuf* amino acid sequences with representatives of eubacteria, archeabacteria, and eukaryotes using neighbor-joining and maximum parsimony methods showed three major clusters representing the three kingdoms of life. Both methods gave similar topologies consistent with the rRNA gene data (data not shown). Within the bacterial clade, the tree is polyphyletic but *tufA* genes from all enterococcal species always clustered with those from other low G+C gram-positive bacteria (except for streptococci and lactococci), while the *tufB* genes of the 11 enterococcal species form a distinct cluster with streptococci and *L. lactis* (Figure 5). Duplicated genes from the same organism do not cluster together, thereby not suggesting evolution by recent gene duplication.

Southern hybridization. Southern hybridization of *Bgl*II/*Xba*I digested genomic DNA from 12 enterococcal species tested with the *tufA* probe (DIG-labeled *tufA* fragment from *E. faecium*) yielded two bands of different sizes in 9 species, which also carried two divergent *tuf* sequences according to their sequencing data. For *E. faecalis and E. solitarius*, a single band was observed indicating that one *tuf* gene is present (Figure 6). A single band was also found when digested genomic DNA from *S. aureus*, *S. pneumoniae*, and *S. pyogenes* were hybridized with the *tufA* probe (data not shown). For *E. faecium*, the presence of three bands can be explained by the existence of a *Xba*I restriction site in the

middle of the *tufA* sequence, which was confirmed by sequencing data. Hybridization with the *tufB* probe (DIG-labeled *tufB* fragment of *E. faecium*) showed a banding profile similar to the one obtained with the *tufA* probe (data not shown).

DISCUSSION

In this study, we have shown that two divergent copies of genes encoding the elongation factor Tu are present in some enterococcal species. Sequence data revealed that both genes are highly conserved at the amino acid level. One copy (*tufA*) is present in all enterococcal species, while the other (*tufB*) is present only in 11 of the 17 enterococcal species studied. Based on 16S rRNA sequence analysis, these 11 species are members of three different enterococcal subgroups (*E. avium*, *E. faecium*, and *E. gallinarum* species groups) and a distinct species (*E. dispar*). Moreover, 16S rDNA phylogeny suggests that these 11 species possessing 2 *tuf* genes all share a common ancestor before they further evolved to become the modern species. Since the six other species having only one copy diverged from the enterococcal lineage before that common ancestor, it appears that the presence of one *tuf* gene in these six species is not attributable to gene loss.

Two clusters of low G+C gram-positive bacteria were observed in the phylogenetic tree of the *tuf* genes: one contains a majority of low G+C gram-positive bacteria and the other contains lactococci and streptococci. This is similar to the finding on the basis of phylogenetic analysis of the 16S rRNA gene and the *hrcA* gene coding for a unique heat-shock regulatory protein. The enterococcal *tufA* genes branched with most of the low G+C gram-positive bacteria, suggesting that they originated from a common ancestor. On the other hand, the enterococcal *tufB* genes branched with the genera *Streptococcus* and *Lactococcus* that form a distinct lineage separated from other low G+C gram-positive bacteria (Figure 5). The finding that these EF-Tu proteins share some conserved amino acid residues unique to this branch also supports the idea that they may share a common ancestor. Although these conserved residues might result from convergent

evolution upon a specialized function, such convergence at the sequence level, even for a few residues, seems to be rare, making it an unlikely event. Moreover, no currently known selective pressure, if any, would account for keeping one versus two *tuf* genes in bacteria. The G+C contents of enterococcal *tufA* and *tufB* sequences are similar, indicating that they both originated from low G+C gram-positive bacteria, in accordance with the phylogenetic analysis.

The *tuf* genes are present in various copy numbers in different bacteria. Furthermore, the two *tuf* genes are normally associated with characteristic flanking genes. The two *tuf* gene copies commonly encountered within gram-negative bacteria are part of the bacterial *str* operon and tRNA-*tufB* operon, respectively. The arrangement of *tufA* in the *str* operon was also found in a variety of bacteria, including *Thermotoga maritima,* the most ancient bacteria sequenced so far, *Aquifex aeolicus*, cyanobacteria, *Bacillus* sp., *Micrococcus luteus*, *Mycobacterium tuberculosis*, and *Streptomyces* sp. Furthermore, the tRNA-*tufB* operon has also been identified in *Aquifex aeolicus*, *Thermus thermophilus*, and *Chlamydia trachomatis*. The two widespread *tuf* gene arrangements argue in favor of their ancient origins. It is noteworthy that most obligate intracellular parasites, such as *Mycoplasma* sp., *R. prowazekii*, *B. burgdorferi*, and *T. pallidum*, contain only one *tuf* gene. Their flanking sequences are distinct from the two conserved patterns as a result of selection for effective propagation by an extensive reduction in genome size by intragenomic recombination and rearrangement.

Most gram-positive bacteria with low G+C content sequenced to date contain only a single copy of the *tuf* gene as a part of the *str* operon. This is the case for *B. subtilis*, *S. aureus* and *E. faecalis*. PCR amplification using a primer targeting a conserved region of the *fus* gene and the *tufA*-specific primer SEQ ID NO. 660, but not the *tufB*-specific primer SEQ ID NO. 661, yielded the expected amplicons for all 17 enterococcal species tested, indicating the presence of the *fus-tuf* organization in all enterococci (data not shown). However, in the genomes of *S. pneumoniae* and *S. pyogenes*, the sequences flanking the *tuf* genes varies although the *tuf* gene itself remains highly conserved. The enterococcal *tufB* genes are

clustered with streptococci, but at present we do not have enough data to identify the genes flanking the enterococcal *tufB* genes. Furthermore, the functional role of the enterococcal *tufB* genes remains unknown. One can only postulate that the two divergent gene copies are expressed under different conditions.

The amino acid sequence identities between the enterococcal *tufA* and *tufB* genes are lower than either i) those between the enterococcal *tufA* and the *tuf* genes from other low G+C gram-positive bacteria (streptococci and lactococci excluded) or ii) those between the enterococcal *tufB* and streptococcal and lactococcal *tuf* genes. These findings suggest that the enterococcal *tufA* genes share a common ancestor with other low G+C gram-positive bacteria via the simple scheme of vertical evolution, while the enterococcal *tufB* genes are more closely related to those of streptococci and lactococci. The facts that some enterococci possess an additional *tuf* gene and that the single streptococcal *tuf* gene is not clustered with other low G+C gram-positive bacteria cannot be explained by the mechanism of gene duplication or intrachromosomal recombination. According to sequence and phylogenetic analysis, we propose that the presence of the additional copy of the *tuf* genes in 11 enterococcal species is due to horizontal gene transfer. The common ancestor of the 11 enterococcal species now carrying *tufB* genes acquired a *tuf* gene from an ancestral streptococcus or a streptococcus-related species during enterococcal evolution through gene transfer before the diversification of modern enterococci. Further study of the flanking regions of the gene may provide more clues for the origin and function of this gene in enterococci.

Recent studies of genes and genomes have demonstrated that considerable horizontal transfer occurred in the evolution of aminoacyl-tRNA synthetases in all three kingdoms of life. The heterogeneity of 16S rRNA is also attributable to horizontal gene transfer in some bacteria, such as *Streptomyces*, *Thermomonospora chromogena* and *Mycobacterium celatum*. In this study, we provide the first example in support of a likely horizontal transfer of the *tuf* gene encoding the elongation factor Tu. This may be an exception since stringent functional constraints do not allow for frequent horizontal transfer of the *tuf* gene as with

other genes. However, enterococcal *tuf* genes should not be the only such exception as we have noticed that the phylogeny of *Streptomyces tuf* genes is equally or more complex than that of enterococci. For example, the three *tuf*-like genes in a high G+C gram-positive bacterium, *S. ramocissimus,* branched with the *tuf* genes of phylogenetically divergent groups of bacteria (Figure 5). Another example may be the *tuf* genes in clostridia, which represent a phylogenetically very broad range of organisms and form a plethora of lines and groups of various complexities and depths. Four species belonging to three different clusters within the genus *Clostridium* have been shown by Southern hybridization to carry two copies of the *tuf* gene. Further sequence data and phylogenetic analysis may help interpreting the evolution of the elongation factor Tu in these gram-positive bacteria. Since the *tuf* genes and 16S rRNA genes are often used for phylogenetic study, the existence of duplicate genes originating from horizontal gene transfer may alter the phylogeny of microorganisms when the laterally acquired copy of the gene is used for such analysis. Hence, caution should be taken in interpreting phylogenetic data. In addition, the two *tuf* genes in enterococci have evolved separately and are distantly related to each other phylogenetically. The enterococcal *tufB* genes are less conserved and unique to the 11 enterococcal species only. We previously demonstrated that the enterococcal *tufA* genes could serve as a target to develop a DNA-based assay for identification of enterococci. The enterococcal *tufB* genes would also be useful in identification of these 11 enterococcal species.

EXAMPLE 43:

<u>Elongation Factor Tu (*tuf*) and the F-ATPase beta-subunit (*atpD*) as phylogenetic tools for species of the family *Enterobacteriaceae.*</u>

SUMMARY

The phylogeny of enterobacterial species commonly found in clinical samples was analyzed by comparing partial sequences of their elongation factor Tu (*tuf*) genes and their F-ATPase beta-subunit (*atpD*) genes. A 884-bp fragment for *tuf* and a 884- or 871-bp fragment for *atpD* were sequenced for 88 strains of 72 species from 25 enterobacterial genera. The *atpD* sequence analysis revealed a specific indel to *Pantoea* and *Tatumella* species showing for the first time a tight phylogenetic affiliation between these two genera. Comprehensive *tuf* and *atpD* phylogenetic trees were constructed and are in agreement with each other. Monophyletic genera are *Yersinia*, *Pantoea*, *Edwardsiella*, *Cedecea*, *Salmonella*, *Serratia*, *Proteus*, and *Providencia*. Analogous trees were obtained based on available 16S rDNA sequences from databases. *tuf* and *atpD* phylogenies are in agreement with the 16S rDNA analysis despite the smaller resolution power for the latter. In fact, distance comparisons revealed that *tuf* and *atpD* genes provide a better resolution for pairs of species belonging to the family *Enterobacteriaceae*. However, 16S rDNA distances are better resolved for pairs of species belonging to different families. In conclusion, *tuf* and *atpD* conserved genes are sufficiently divergent to discriminate different species inside the family *Enterobacteriaceae* and offer potential for the development of diagnostic tests based on DNA to identify enterobacterial species.

INTRODUCTION

Members of the family *Enterobacteriaceae* are facultatively anaerobic gram-negative rods, catalase-positive and oxydase-positive (Brenner, 1984). They are found in soil, water, plants, and in animals from insects to man. Many enterobacteria are opportunistic pathogens. In fact, members of this family are responsible for about 50 % of nosocomial infections in the United States (Brenner, 1984). Therefore, this family is of considerable clinical importance.

Major classification studies on the family *Enterobacteriaceae* are based on phenotypic traits (Brenner *et al.*, 1999; Brenner *et al.*, 1980; Dickey & Zumoff,

1988; Farmer III *et al.*, 1980; Farmer III *et al.*, 1985b; Farmer III *et al.*, 1985a) such as biochemical reactions and physiological characteristics. However, phenotypically distinct strains may be closely related by genotypic criteria and may belong to the same genospecies (Bercovier *et al.*, 1980; Hartl & Dykhuizen, 1984). Also, phenotypically close strains (biogroups) may belong to different genospecies, like *Klebsiella pneumoniae* and *Enterobacter aerogenes* (Brenner, 1984) for example. Consequently, identification and classification of certain species may be ambiguous with techniques based on phenotypic tests (Janda *et al.*, 1999; Kitch *et al.*, 1994; Sharma *et al.*, 1990).

More advances in the classification of members of the family *Enterobacteriaceae* have come from DNA-DNA hybridization studies (Brenner *et al.*, 1993; Brenner *et al.*, 1986; Brenner, *et al.*, 1980; Farmer III, *et al.*, 1980; Farmer III, *et al.*, 1985b; Izard *et al.*, 1981; Steigerwalt *et al.*, 1976). Furthermore, the phylogenetic significance of bacterial classification based on 16S rDNA sequences has been recognized by many workers (Stackebrandt & Goebel, 1994; Wayne *et al.*, 1987). However, members of the family *Enterobacteriaceae* have not been subjected to extensive phylogenetic analysis of 16S rDNA (Sproer *et al.*, 1999). In fact, this molecule was not thought to solve taxonomic problems concerning closely related species because of its very high degree of conservation (Brenner, 1992; Sproer, *et al.*, 1999). Another drawback of the 16S rDNA gene is that it is found in several copies within the genome (seven in *Escherichia coli* and *Salmonella typhimurium*) (Hill & Harnish, 1981). Due to sequence divergence between the gene copies, direct sequencing of PCR products is often not suitable to achieve a representative sequence (Cilia *et al.*, 1996; Hill & Harnish, 1981). Other genes such as *gap* and *ompA* (Lawrence *et al.*, 1991), *rpoB* (Mollet *et al.*, 1997), and *infB* (Hedegaard *et al.*, 1999) were used to resolve the phylogeny of enterobacteria. However, none of these studies covered an extensive number of species.

tuf and *atpD* are the genes encoding the elongation factor Tu (EF-Tu) and the F-ATPase beta-subunit, respectively. EF-Tu is involved in peptide chain formation (Ludwig *et al.*, 1990). The two copies of the *tuf* gene (*tufA* and *tufB*) found in enterobacteria (Sela *et al.*, 1989) share high identity level (99 %) in *Salmonella typhimurium* and in *E. coli*. The recombination phenomenon could explain sequence homogenization between the two copies (Abdulkarim & Hughes, 1996; Grunberg-Manago, 1996). F-ATPase is present on the plasma membranes of eubacteria (Nelson & Taiz, 1989). It functions mainly in ATP synthesis (Nelson & Taiz, 1989) and the beta-subunit contains the catalytic site of the enzyme. EF-Tu and F-ATPase are highly conserved throughout evolution and shows functional constancy (Amann *et al.*, 1988; Ludwig, *et al.*, 1990). Recently, phylogenies based on protein sequences from EF-Tu and F-ATPase beta-subunit showed good agreement with each other and with the rDNA data (Ludwig *et al.*, 1993).

We elected to sequence 884-bp fragments of *tuf* and *atpD* from 88 clinically relevant enterobacterial strains representing 72 species from 25 genera. These sequences were used to create phylogenetic trees that were compared with 16S rDNA trees. These trees revealed good agreement with each others and demonstrated the high resolution of *tuf* and *atpD* phylogenies at the species level.

MATERIALS AND METHODS

Bacterial strains and genomic material. All bacterial strains used in this study were obtained from the American Type Culture Collection (ATCC) or the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ). These enterobacteria can all be recovered from clinical specimens, but not all are pathogens. Whenever possible, we choose type strains. Identification of all strains was confirmed by classical biochemical tests using the automated system MicroScan WalkAway-96 system equipped with a Negative BP Combo Panel Type 15 (Dade Behring Canada). Genomic DNA was purified using the G NOME

DNA kit (Bio 101). Genomic DNA from *Yersinia pestis* was kindly provided by Dr. Robert R. Brubaker. Strains used in this study and their descriptions are shown in Table 19.

PCR primers. The eubacterial *tuf* and *atpD* gene sequences available from public databases were analyzed using the GCG package (version 8.0) (Genetics Computer Group). Based on multiple sequence alignments, two highly conserved regions were chosen for each genes, and PCR primers were derived from these regions with the help of Oligo primer analysis software (version 5.0) (National Biosciences). A second 5' primer was design to amplify the gene *atpD* for few enterobacteria difficult to amplifiy with the first primer set. When required, the primers contained inosines or degeneracies to account for variable positions. Oligonucleotide primers were synthesized with a model 394 DNA/RNA synthesizer (PE Applied Biosystems). PCR primers used in this study are listed in Table 20.

DNA sequencing. An 884-bp portion of the *tuf* gene and an 884-bp portion (or alternatively an 871-bp portion for a few enterobacterial strains) of the *atpD* gene were sequenced for all enterobacteria listed in the first strain column of Table 19. Amplification was performed with 4 ng of genomic DNA. The 40-μl PCR mixtures used to generate PCR products for sequencing contained 1·0 μM each primer, 200 μM each deoxyribonucleoside triphosphate (Pharmacia Biotech), 10 mM Tris-HCl (pH 9·0 at 25 °C), 50 mM KCl, 0·1 % (w/v) Triton X-100, 2·5 mM $MgCl_2$, 0·05 mM BSA, 0·3 U of *Taq* DNA polymerase (Promega) coupled with TaqStart™ antibody (Clontech Laboratories). The TaqStart™ neutralizing monoclonal antibody for *Taq* DNA polymerase was added to all PCR mixtures to enhance efficiency of amplification (Kellogg *et al.*, 1994). The PCR mixtures were subjected to thermal cycling (3 min at 95 °C and then 35 cycles of 1 min at 95 °C, 1 min at 55 °C for *tuf* or 50 °C for *atpD*, and 1 min at 72 C, with a 7-min final extension at 72 °C) using a PTC-200 DNA Engine thermocycler (MJ Research).

PCR products having the predicted sizes were recovered from an agarose gel stained for 15 min with 0·02 % of methylene blue followed by washing in sterile distilled water for 15 min twice (Flores *et al.*, 1992). Subsequently, PCR products having the predicted sizes were recovered from gels using the QIAquick gel extraction kit (QIAGEN).

Both strands of the purified amplicons were sequenced using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems) on an automated DNA sequencer (Model 377). Amplicons from two independant PCR amplifications were sequenced for each strain to ensure the absence of sequencing errors attributable to nucleotide miscorporations by the *Taq* DNA polymerase. Sequence assembly was performed with the aid of Sequencher 3.0 software (Gene Codes).

Phylogenetic analysis. Multiple sequence alignments were performed using PileUp from the GCG package (Version 10.0) (Genetics Computer Group) and checked by eye with the editor SeqLab to edit sequences if necessary and to note which regions were to be excluded for phylogenetic analysis. *Vibrio cholerae* and *Shewanella putrefaciens* were used as outgroups. Bootstrap subsets (750 sets) and phylogenetic trees were generated with the Neighbor Joining algorithm from Dr. David Swofford's PAUP (Phylogenetic Analysis Using Parsimony) Software version 4.0b4 (Sinauer Associates) and with tree-bisection branch-swapping. The distance model used was Kimura (1980) two-parameter. Relative rate test was performed with the aid of Phyltest program version 2.0 (c).

RESULTS AND DISCUSSION

DNA amplification, sequencing and sequence alignments

A PCR product of the expected size of 884 bp was obtained for *tuf* and of 884 or 871 bp for *atpD* from all bacterial strains tested. After subtracting for biased

primer regions and ambiguous single strand data, sequences of at least 721 bp for *tuf* and 713 bp for *atpD* were submitted to phylogenetic analyses. These sequences were aligned with *tuf* and *atpD* sequences available in databases to verify that the nucleotide sequences indeed encoded a part of tested genes. Gaps were excluded to perform phylogenetic analysis.

Signature sequences

From the sequence alignments obtained from both tested genes, only one insertion was observed. This five amino acids insertion is located between the positions 325 and 326 of *atpD* gene of *E. coli* strain K-12 (Saraste *et al.*, 1981) and can be considered a signature sequence of *Tatumella ptyseos* and *Pantoea* species (Fig. 7). The presence of a conserved indel of defined length and sequence and flanked by conserved regions could suggest a common ancestor, particularly when members of a given taxa share this indel (Gupta, 1998). To our knowledge, high relatedness between the genera *Tatumella* and *Pantoea* is demonstrated for the first time.

Enterobacter agglomerans ATCC 27989 sequence does not possess the five amino acid indel (Fig. 7). This indel could represent a useful marker to help resolve the *Enterobacter agglomerans* and *Pantoea* classification. Indeed, the transfer of *Enterobacter agglomerans* to *Pantoea agglomerans* was proposed in 1989 by Gavini *et al.* (Gavini *et al.*, 1989). However, some strains are provisionally classified as *Pantoea* sp. until their interrelatedness is elucidated (Gavini, *et al.*, 1989). Since the transfer was proposed, the change of nomenclature has not yet been made for all *Enterobacter agglomerans* in the ATCC database. The absence of the five amino acids indel suggests that some strains of *Enterobacter agglomerans* most likely do not belong to the genus *Pantoea*.

Phylogenetic trees based on partial *tuf* sequences, atpD sequences, and published 16S rDNA data of members of the *Enterobacteriaceae*.

Representative trees constructed from *tuf* and *atpD* sequences with the neighbor-joining method are shown in Fig. 8. The phylogenetic trees generated from partial *tuf* sequences and *atpD* sequences are very similar. Nevertheless, *atpD* tree shows more monophyletic groups corresponding to species that belong to the same genus. These groups are more consistent with the actual taxonomy. For both genes, some genera are not monophyletic. These results support previous phylogenies based on the genes *gap* and *ompA* (Lawrence, *et al.*, 1991), *rpoB* (Mollet, *et al.*, 1997), and *infB* (Hedegaard, *et al.*, 1999) which all showed that the genera *Escherichia* and *Klebsiella* are polyphyletic. There were few differences in branching between *tuf* and *atpD* genes.

Even though *Pantoea agglomerans* and *Pantoea dispersa* indels were excluded for phylogenetic analysis, these two species grouped together and were distant from *Enterobacter agglomerans* ATCC 27989, adding another evidence that the latter species is heterogenous and that not all members of this species belong to the genus *Pantoea*. In fact, the *E. agglomerans* strain ATCC 27989 exhibits branch lengths similar to others *Enterobacter* species with both genes. Therefore, we suggest that this strain belong to the genus *Enterobacter* until further reclassification of that genus.

tuf and *atpD* trees exhibit very short genetic distances between taxa belonging to the same genetic species including species segregated for clinical considerations. This first concern *E. coli* and *Shigella* species that were confirmed to be the same genetic species by hybridization studies (Brenner *et al.*, 1972; Brenner *et al.*, 1972; Brenner *et al.*, 1982) and phylogenies based on 16S rDNA (Wang *et al.*, 1997) and *rpoB* genes (Mollet, *et al.*, 1997). Hybridization studies (Bercovier, *et al.*, 1980) and phylogeny based on 16S rDNA genes (Ibrahim *et al.*, 1994) demonstrated also that *Yersinia pestis* and *Y. pseudotuberculosis* are the same genetic species. Among

Yersinia pestis and *Y. pseudotuberculosis,* the three *Klebsiella pneumoniae* subspecies, *E. coli-Shigella* species, and *Salmonella choleraesuis* subspecies, *Salmonella* is a less tightly knit species than the other genetic species. The same is true for *E. coli* and *Shigella* species.

Escherichia fergusonii is very close to *E. coli-Shigella* genetic species. This observation is corroborated by 16S rDNA phylogeny (McLaughlin *et al.*, 2000) but not by DNA hybridization values. In fact, *E. fergusonii* is only 49% to 63% related to *E. coli-Shigella* (Farmer III, *et al.*, 1985b). It was previously observed that very recently diverged species may not be recognizable based on 16S rDNA sequences although DNA hybridization established them as different species (Fox *et al.*, 1992). Therefore, *E. fergusonii* could be a new "quasi-species".

atpD phylogeny revealed *Salmonella* subspecies divisions consistent with the actual taxonomy. This result was already observed by Christensen *et al.* (Christensen & Olsen, 1998). Nevertheless, *tuf* partial sequences discriminate less than *atpD* between *Salmonella* subspecies.

Overall, *tuf* and *atpD* phylogenies exhibit enough divergence between species to ensure efficient discrimination. Therefore, it could be easy to distinguish phenotypically close enterobacteria belonging to different genetic species such as *Klebsiella pneumoniae* and *Enterobacter aerogenes.*

Phylogenetic relationships between *Salmonella, E. coli* and *C. freundii* are not well defined. 16S rDNA and 23S rDNA sequence data reveals a closer relationship between *Salmonella* and *E. coli* than between *Salmonella* and *C. freundii* (Christensen *et al.*, 1998), while DNA homology studies (Selander *et al.*, 1996) and *infB* phylogeny (Hedegaard, *et al.*, 1999) showed that *Salmonella* is more closely related to *C. freundii* than to *E. coli.* In that regard, *tuf* and *atpD* phylogenies are coherent with 16S rDNA and 23S rDNA sequence analysis.

Phylogenetic analyses were also performed using amino acids sequences. *tuf* tree based on amino acids is characterized by a better resolution between taxa outgroup and taxa ingroup (enterobacteria) than tree based on nucleic acids whereas *atpD* trees based on amino acids and nucleic acids give almost the same resolution between taxa outgroup and ingroup (data not shown).

Relative rate test (or two cluster test (Takezaki *et al.*, 1995)) evaluates if evolution is constant between two taxa. Before to apply the test, the topology of a tree is determined by tree-building method without the assumption of rate constancy. Therefore, two taxa (or two groups of taxa) are compared with a third taxon that is an outgroup of the first two taxa (Takezaki, *et al.*, 1995). Few pairs of taxa that exhibited a great difference between their branch lengths at particular nodes were chosen to perform the test. This test reveals that *tuf* and *atpD* are not constant in their evolution within the family *Enterobacteriaceae*. For *tuf*, for example, the hypothesis of rate constancy is rejected (Z value higher than 1·96) between *Yersinia* species. The same is true for *Proteus* species. For *atpD*, for example, evolution is not constant between *Proteus* species, between *Proteus* species and *Providencia* species, and between *Yersinia* species and *Escherichia coli*. For 16S rDNA, for example, evolution is not constant between two *E. coli*, between *E. coli* and *Enterobacter aerogenes*, and between *E. coli* and *Proteus vulgaris*. These results suggest that *tuf*, *atpD* and 16S rDNA could not serve as a molecular clock for the entire family *Enterobacteriaceae*.

Since the number and the nature of taxa can influence topology of trees, phylogenetic trees from *tuf* and *atpD* were reconstructed using sequences corresponding to strains for which 16S rDNA genes were published in GenEMBL. These trees were similar to those generated using 16S rDNA (Fig. 9). Nevertheless, 16S rDNA tree gave poorer resolution power than *tuf* and *atpD* gene trees. Indeed, these latter exhibited less multifurcation (polytomy) than the 16S rDNA tree.

Comparison of distances based on *tuf*, *atpD*, and 16S rDNA data.

tuf, *atpD*, and 16S rDNA distances (i.e. the number of differences per nucleotide site) were compared with each other for each pair of strains. We found that the *tuf* and *atpD* distances were respectively 2·268 ± 0·965 and 2·927 ± 0·896 times larger than 16S rDNA distances (Fig. 10a and b). *atpD* distances were 1·445 ± 0·570 times larger than *tuf* distances (Fig. 10c). Figure 10 also shows that the *tuf*, *atpD*, and 16S rDNA distances between members of different species of the same genus (0·053 ± 0·034, 0·060 ± 0·020, and 0·024 ± 0·010, respectively) were in mean smaller than the distances between members of different genera belonging to the same family (0·103 ± 0·053, 0·129 ± 0·051, and 0·044 ± 0·013, respectively). However, the overlap exhibits with standard deviations add to a focus of evidences that some enterobacterial genera are not well defined (Brenner, 1984). In fact, many distances for pairs of species especially belonging to the genera *Escherichia*, *Shigella*, *Enterobacter*, *Citrobacter*, *Klebsiella*, and *Kluyvera* overlap distances for pairs of species belonging to the same genus (Fig. 10). For example, distances for pairs composed by species of *Citrobacter* and species of *Klebsiella* overlap distances for pairs composed by two *Citrobacter* or by two *Klebsiella*.

Observing the distance distributions, 16S rDNA distances reveal a clear separation between the families *Enterobacteriaceae* and *Vibrionaceae* despite the fact that the family *Vibrionaceae* is genetically very close to the *Enterobacteriaceae* (Fig. 10a and b). Nevertheless, *tuf* and *atpD* show higher discriminating power below the family level (Fig. 10a and b).

There were some discrepancies in the relative distances for the same pairs of taxa between the two genes studied. First, distances between *Yersinia* species are at least two times lower for *atpD* than for tuf (Fig. 10c). Also, distances at the family level (between *Enterobacteriaceae* and *Vibrionaceae*) show that *Enterobacteriaceae* is a tightlier knit family with *atpD* gene (Proteus genus

excepted) than with *tuf* gene. Both genes well delineate taxa belonging to the same species. There is one exception with *atpD*: *Klebsiella planticola* and *K. ornithinolithica* belong to the same genus but fit with taxa belonging to the same species (Fig. 10a and c). These two species are also very close genotypically with *tuf* gene. This suggest that *Klebsiella planticola* and *K. ornithinolithica* could be two newborn species. *tuf* and *atpD* genes exhibit little distances between *Escherichia fergusonii* and *E. coli-Shigella* species. Unfortunately, comparison with 16S rDNA could not be achieved because the *E. fergusonii* 16S rDNA sequence is not yet accessible in GenEMBL database. Therefore, the majority of phenotypically close enterobacteria could be easily discriminated genotypically using *tuf* and *atpD* gene sequences.

In conclusion, *tuf* and *atpD* genes exhibit phylogenies consistent with 16S rDNA genes phylogeny. For example, they reveal that the family *Enterobacteriaceae* is monophyletic. Moreover, *tuf* and *atpD* distances provide a higher discriminating power than 16S rDNA distances. In fact, *tuf* and *atpD* genes discriminate well between different genospecies and are conserved between strains of the same genetic species in such a way that primers and molecular probes for diagnostic purposes could be designed. Preliminary studies support these observations and diagnostic tests based on *tuf* and *atpD* sequence data to identify enterobacteria are currently under development.

EXAMPLE 44:

<u>Testing new pairs of PCR primers selected from two species-specific genomic DNA fragments which are objects of our assigned US patent 6,001,564</u>

<u>Objective</u>: The goal of these experiments is to demonstrate that it is relatively easy for a person skilled in the art to find other PCR primer pairs from the species-specific

fragments used as targets for detection and identification of a variety of microorganisms. In fact, we wish to prove that the PCR primers previously tested by our group and which are objects of the present patent application are not the only possible good choices for diagnostic purposes. For this example, we used diagnostic targets described in our assigned US patent 6,001,564.

Experimental strategy: We have selected randomly two species-specific genomic DNA fragments for this experiment. The first one is the 705-bp fragment specific to *Staphylococcus epidermidis* (SEQ ID NO: 36 from US patent 6,001,564) while the second one is the 466-bp fragment specific to *Moraxella catarrhalis* (SEQ ID NO: 29 from US patent 6,001,564). Subsequently, we have selected from these two fragments a number of PCR primer pairs other than those previously tested. We have chosen 5 new primer pairs from each of these two sequences which are well dispersed along the DNA fragment (Figures 11 and 12). We have tested these primers for their specificity and compared them with the original primers previously tested. For the specificity tests, we have tested all bacterial species closely related to the target species based on phylogenetic analysis with three conserved genes (rRNA genes, *tuf* and *atpD*). The rational for selecting a restricted number of bacterial species to evaluate the specificity of the new primer pairs is based on the fact that the lack of specificity of a DNA-based assay is attributable to the detection of closely related species which are more similar at the nucleotide level. Based on the phylogenetic analysis, we have selected (i) species from the closely related genus *Staphylococcus*, *Enterococcus*, *Streptococcus* and *Listeria* to test the specificity of the *S. epidermidis*-specific PCR assays and (ii) species from the closely related genus *Moraxella*, *Kingella* and *Neisseria* to test the specificity of the *M. catarrhalis*-specific PCR assays.

Materials and methods

Bacterial strains. All bacterial strains used for these experiments were obtained from the American Type Culture Collection (ATCC, Rockville, MD).

Genomic DNA isolation. Genomic DNA was purified from the ATCC reference strains by using the G-nome DNA kit (Bio 101 Inc., Vista, CA).

Oligonucleotide design and synthesis. PCR primers were designed with the help of the Oligo™ primer analysis software Version 4.0 (National Biosciences Inc., Plymouth, Minn.) and synthesized using a model 391 DNA synthesizer (Applied Biosystems, Foster City, CA).

PCR assays. All PCR assays were performed by using genomic DNA purified from reference strains obtained from the ATCC. One μl of purified DNA preparation (containing 0.01 to 1 ng of DNA per μl) was added directly into the PCR reaction mixture. The 20 μL PCR reactions contained final concentrations of 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM $MgCl_2$, 0.4 μM of each primer, 200 μM of each of the four dNTPs and 0.5 unit of *Taq* DNA polymerase (Promega, Madison, WI) combined with the TaqStart™ antibody (Clontech Laboratories Inc., Palo Alto, CA). An internal control was integrated into all amplification reactions to verify the efficiency of the amplification reaction as well as to ensure that significant PCR inhibition was absent. Primers amplifying a region of 252 bp from a control plasmid added to each amplification reaction were used to provide the internal control. PCR reactions were then subjected to thermal cycling (3 min at 95°C followed by 30 cycles of 1 second at 95°C for the denaturation step and 30 seconds at 50 to 65°C for the annealing-extension step) using a PTC-200 thermal cycler (MJ Research Inc., Watertown, MA). PCR amplification products were then analyzed by standard agarose gel (2%) electrophoresis. Amplification products were visualized in agarose gels containing 0.25 μg/mL of ethidium bromide under UV at 254 nm.

Results

Tables 21 and 22 show the results of specificity tests with the 5 new primer pairs selected from SEQ ID NO: 29 (specific to *M. catarrhalis* from US patent 6,001,564) and SEQ ID NO: 36 (specific to *S. epidermidis* from US patent 6,001,564), respectively. In order to evaluate the performance of these new primers pairs, we compared them in parallel with the original primer pairs previously tested.

For *M. catarrhalis*, all of the 5 selected PCR primer pairs were specific for the target species because none of the closely related species could be amplified (Table 21). In fact, the comparison with the original primer pair SEQ ID NO: 118 + SEQ ID NO: 119 (from US patent 6,001,564) revealed that all new pairs showed identical results in terms of specificity and sensitivity thereby suggesting their suitability for diagnostic purposes.

For *S. epidermidis*, 4 of the 5 selected PCR primer pairs were specific for the target species (Table 22). It should be noted that for 3 of these four primer pairs the annealing temperature had to be increased from 55 °C to 60 or 65 °C to attain specificity for *S. epidermidis*. Again the comparison with the original primer pair SEQ ID NO: 145 + SEQ ID NO: 146 (from US patent 6,001,564) revealed that these four primer pairs were as good as the original pair. Increasing the annealing temperature for the PCR amplification is well known by persons skilled in the art to be a very effective way to improve the specificity of a PCR assay (Persing *et al.*, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.; Ehrlich and Greenberg, 1994, PCR-based Diagnostics in Infectious Disease, Blackwell Scientific Publications, Boston, MA). In fact, those skilled in the art are well aware of the fact that the annealing temperature is critical for the optimization of PCR assays. Only the primer pair VBsep3 + VBsep4 amplified bacterial species other than *S. epidermidis* including the staphylococcal species *S. capitis, S. cohnii, S. aureus, S. haemolyticus* and *S. hominis* (Table 22). For this non-specific primer pair, increasing the annealing temperature

from 55 to 65 °C was not sufficient to attain the desired specificity. One possible explanation for the fact that it appears sligthly easier to select species-specific primers for *M. catarrhalis* than for *S. epidermidis* is that *M. catarrhalis* is more isolated in phylogenetic trees than *S. epidermidis*. The large number of coagulase negative staphylococcal species such as *S. epidermidis* is largely responsible for this phylogenetic clustering.

Conclusion

These experiment clearly show that it is relatively easy for a person skilled in the art to select, from the species-specific DNA fragments selected as target for identification, PCR primer pairs suitable for diagnostic purposes other than those previously tested. The amplification conditions can be optimize by modifying critical variables such as the annealing temperature to attain the desired specificity and sensitivity. Consequently, we consider that it is legitimate to claim any possible primer sequences selected from the species-specific fragment and that it would be unfair to grant only the claims dealing with the primer pairs previously tested. By extrapolation, these results strongly suggest that it is also relatively easy for a person skilled in the art to select, from the species-specific DNA fragments, DNA probes suitable for diagnostic purposes other than those previously tested.

EXAMPLE 45:

Testing modified versions of PCR primers derived from the sequence of several primers which are objects of US patent 6,001,564.

Objective: The purpose of this project is to verify the efficiency of amplification by modified PCR primers derived from primers previously tested. The types of primer modifications to be tested include (i) variation of the sequence at one or more nucleotide positions and (ii) increasing or reducing the length of the primers. For this example, we used diagnostic targets described in US patent 6,001,564.

Experimental strategy:

a) Testing primers with nucleotide changes

We have designed 13 new primers which are derived from the *S. epidermidis*-specific SEQ ID NO: 146 from US patent 6,001,564 (Table 23). These primers have been modified at one or more nucleotide positions. As shown in Table 23, the nucleotide changes were introduced all along the primer sequence. Furthermore, instead of modifying the primer at any nucleotide position, the nucleotide changes were introduced at the third position of each codon to better reflect potential genetic variations *in vivo*. It should be noted that no nucleotide changes were introduced at the 3' end of the oligonucleotide primers because those skilled in the art are well aware of the fact that mimatches at the 3' end should be avoided (Persing *et al.*, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.). All of these modified primers were tested in PCR assays in combination with SEQ ID NO: 145 from US patent 6,001,564 and the efficiency of the amplification was compared with the original primer pair SEQ ID NO: 145 + SEQ ID NO: 146 previously tested in US patent 6,001,564.

b) Testing shorter or longer versions of primers

We have designed shorter and longer versions of the original *S. epidermidis*-specific PCR primer pair SEQ ID NO: 145 + 146 from US patent 6,001,564 (Table 24) as well as shorter versions of the original *P. aeruginosa*-specific primer pair SEQ ID NO: 83 + 84 from US patent 6,001,564 (Table 25). As shown in Tables 24 and 25, both primers of each pair were shortened or lengthen to the same length. Again, those skilled in the art know that the melting temperature of both primers from a pair should be similar to avoid preferential binding at one primer binding site which is

detrimental in PCR (Persing *et al.*, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.; Ehrlich and Greenberg, 1994, PCR-based Diagnostics in Infectious Disease, Blackwell Scientific Publications, Boston, MA). All of these shorter or longer primer versions were tested in PCR assays and the efficiency of the amplification was compared with the original primer pair SEQ ID NOs 145 and 146.

Materials and methods

See the Materials and methods section of Example 44.

Results

a) Testing primers with nucleotide changes

The results of the PCR assays with the 13 modified versions of SEQ ID NO: 146 from US patent 6,001,564 are shown in Table 23. The 8 modified primers having a single nucleotide variation showed an efficiency of amplification identical to the original primer pair based on testing with 3 different dilutions of genomic DNA. The four primers having two nucleotide variations and primer VBmut12 having 3 nucleotide changes also showed PCR results identical to those obtained with the original pair. Finally, primer VBmut13 with four nucleotide changes showed a reduction in sensitivity by approximately one log as compared with the original primer pair. However, reducing the annealing temperature from 55 to 50 °C gave an efficiency of amplification very similar to that observed with the original primer pair (Table 23). In fact, reducing the annealing temperature of PCR cycles represents an effective way to reduce the stringency of hybridization for the primers and consequently allows the binding of probes with mismatches (Persing *et al.*, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.). Subsequently, we have confirmed the

specificity of the PCR assays with each of these 13 modified versions of SEQ ID NO: 146 from US patent 6,001,564 by performing amplifications from all bacterial species closely related to *S. epidermidis* which are listed in Table 22.

b) Testing shorter or longer versions of primers

For these experiments, two primer pairs were selected: i) SEQ ID NO: 145 + 146 from US patent 6,001,564 (specific to *S. epidermidis*) which are AT rich and ii) SEQ ID NO: 83 + 84 (specific to *P. aeruginosa*) which are GC rich. For the AT rich sequence, primers of 15 to 30 nucleotide in length were designed (Table 24) while for the GC rich sequences, primers of 13 to 19 nucleotide in length were designed (Table 25).

Table 24 shows that, for an annealing temperature of 55 °C, the 30- 25-, 20- and 17-nucleotide versions of SEQ ID NO: 145 and 146 from US patent 6,001,564 all showed identical results as compared with the original primer pair except that the 17-nucleotide version amplified slightly less efficiently the *S. epidermidis* DNA. Reducing the annealing temperature from 55 to 45 °C for the 17-nucleotide version allowed to increase the amplification efficiency to a level very similar to that with the original primer pair (SEQ ID NO: 145 + 146 from US patent 6,001,564). Regarding the 15-nucleotide version, there was amplification *of S. epidermidis* DNA only when the annealing temperature was reduced to 45 °C. Under those PCR conditions the assay remained *S. epidermidis*-specific but the amplification signal with *S. epidermidis* DNA was sligthly lower as compared with the original primer pair. Subsequently, we have further confirmed the specificity of the shorter or longer versions by amplifying DNA from all bacterial species closely related to *S. epidermidis* which are listed in Table 22.

Table 25 shows that, for an annealing temperature of 55 °C, all shorter versions of SEQ ID NO: 83 and 84 from US patent 6,001,564 showed identical PCR results as

compared with the original primer pair. As expected, these results show that it is simpler to reduce the length of GC rich as compared with AT rich. This is attributable to the fact that GC binding is more stable than AT binding.

Conclusion

a) Testing primers with nucleotide changes

The above experiments clearly show that PCR primers may be modified at one or more nucleotide positions without affecting the specificity and the sensitivity of the PCR assay. These results strongly suggest that a given oligonucleotide can detect variant genomic sequences from the target species. In fact, the nucleotide changes in the selected primers were purposely introduced at the third position of each codon to mimic nucleotide variation in genomic DNA. Thus we conclude that it is justified to claim "a variant thereof" for i) the SEQ IDs of the fragments and oligonucleotides which are object of the present patent application and ii) genomic variants of the target species.

b) Testing shorter or longer versions of primers

The above experiments clearly show that PCR primers may be shorter or longer without affecting the specificity and the sensitivity of the PCR assay. We have showed that oligonucleotides ranging in sizes from 13 to 30 nucleotides may be as specific and sensitive as the original primer pair from which they were derived. Consequently, these results suggest that it is not exaggerated to claim sequences having at least 12 nucleotide in length.

This invention has been described herein above, and it is readily apparent that modifications can be made thereto without departing from the spirit of this invention. These modifications are under the scope of this invention, as defined in the appended claims.

Table 1. Distribution (%) of nosoc mial path gens for vari us human infections in USA (1990-1992)[1].

Pathogen	UTI[2]	SSI[3]	BSI[4]	Pneumonia	CSF[5]
Escherichia coli	27	9	5	4	2
Staphylococcus aureus	2	21	17	21	2
Staphylococcus epidermidis	2	6	20	0	1
Enterococcus faecalis	16	12	9	2	0
Enterococcus faecium	1	1	0	0	0
Pseudomonas aeruginosa	12	9	3	18	0
Klebsiella pneumoniae	7	3	4	9	0
Proteus mirabilis	5	3	1	2	0
Streptococcus pneumoniae	0	0	3	1	18
Group B *Streptococci*	1	1	2	1	6
Other streptococci	3	5	2	1	3
Haemophilus influenzae	0	0	0	6	45
Neisseria meningitidis	0	0	0	0	14
Listeria monocytogenes	0	0	0	0	3
Other enterococci	1	1	0	0	0
Other staphylococci	2	8	13	2	0
Candida albicans	9	3	5	5	0
Other *Candida*	2	1	3	1	0
Enterobacter sp.	5	7	4	12	2
Acinetobacter sp.	1	1	2	4	2
Citrobacter sp.	2	1	1	1	0
Serratia marcescens	1	1	1	3	1
Other *Klebsiella*	1	1	1	2	1
Others	0	6	4	5	0

[1] Data recorded by the National Nosocomial Infections Surveillance (NNIS) from 80 hospitals (Emori and Gaynes, 1993, *Clin. Microbiol. Rev.*, **6**:428-442).

[2] Urinary tract infection.

[3] Surgical site infection.

[4] Bloodstream infection.

[5] Cerebrospinal fluid.

Tabl 2. Distribution (%) f bloodstr am infecti n pathogens in Qu bec (1995), Canada (1992), UK (1969-1988) and USA (1990-1992).

Organism	Quebec[1]	Canada[2]	UK[3] Community-acquired	UK[3] Hospital-acquired	USA[4] Hospital-acquired
E. coli	15.6	53.8	24.8	20.3	5.0
S. epidermidis and other CoNS[5]	25.8	-	0.5	7.2	31.0
S. aureus	9.6	-	9.7	19.4	16.0
S. pneumoniae	6.3	-	22.5	2.2	-
E. faecalis	3.0	-	1.0	4.2	-
E. faecium	2.6	-	0.2	0.5	-
Enterococcus sp.	-	-	- -	9.0	
H. influenzae	1.5	-	3.4	0.4	-
P. aeruginosa	1.5	8.2	1.0	8.2	3.0
K. pneumoniae	3.0	11.2	3.0	9.2	4.0
P. mirabilis	-	3.9	2.8	5.3	1.0
S. pyogenes	-	-	1.9	0.9	-
Enterobacter sp.	4.1	5.5	0.5	2.3	4.0
Candida sp.	8.5	-	-	1.0	8.0
Others	18.5	17.4	28.7	18.9	19.0

[1] Data obtained for 270 isolates collected at the Centre Hospitalier de l'Université Laval (CHUL) during a 5 month period (May to October 1995).

[2] Data from 10 hospitals throughout Canada representing 941 gram-negative isolates. (Chamberland *et al.*, 1992, *Clin. Infect. Dis.*, **15**:615-628).

[3] Data from a 20-year study (1969-1988) for nearly 4000 isolates. (Eykyn *et al.*, 1990, *J. Antimicrob. Chemother.*, Suppl. C, **25**:41-58).

[4] Data recorded by the National Nosocomial Infections Surveillance (NNIS) from 80 hospitals (Emori and Gaynes, 1993, *Clin. Microbiol. Rev.*, **6**:428-442).

[5] Coagulase-negative staphylococci.

Tabl 3. Distributi n f positive and negativ clinical specimens tested at th micr biology laboratory of the CHUL (February 1994 – January 1995).

Clinical specimens and/or sites	No. of samples tested (%)	% of positive specimens	% of negative specimens
Urine	17,981 (54.5)	19.4	80.6
Blood culture/marrow	10,010 (30.4)	6.9	93.1
Sputum	1,266 (3.8)	68.4	31.6
Superficial pus	1,136 (3.5)	72.3	27.7
Cerebrospinal fluid	553 (1.7)	1.0	99.0
Synovial fluid	523 (1.6)	2.7	97.3
Respiratory tract	502 (1.5)	56.6	43.4
Deep pus	473 (1.4)	56.8	43.2
Ears	289 (0.9)	47.1	52.9
Pleural and pericardial fluid	132 (0.4)	1.0	99.0
Peritoneal fluid	101(0.3)	28.6	71.4
Total:	**32,966 (100.0)**	**20.0**	**80.0**

Table 4. **Example of microbial species for which *tuf* and/or *atpD* and/or *recA* nucleic acids and/or sequences are used in the present invention.**

Bacterial species

Abiotrophia adiacens
Abiotrophia defectiva
Achromobacter xylosoxidans subsp. *denitrificans*
Acetobacterium woodi
Acetobacter aceti
Acetobacter altoacetigenes
Acetobacter polyoxogenes
Acholeplasma laidlawii
Acidothermus cellulolyticus
Acidiphilum facilis
Acinetobacter baumannii
Acinetobacter calcoaceticus
Acinetobacter lwoffii
Actinomyces meyeri
Aerococcus viridans
Aeromonas hydrophila
Aeromonas salmonicida
Agrobacterium radiobacter
Agrobacterium tumefaciens
Alcaligenes faecalis *subsp.* faecalis
Allochromatium vinosum
Anabaena variabilis
Anacystis nidulans
Anaerorhabdus furcosus
Aquifex aeolicus
Aquifex pyrophilus
Arcanobacterium haemolyticum
Archaeoglobus fulgidus
Azotobacter vinelandii
Bacillus anthracis
Bacillus cereus
Bacillus firmus
Bacillus halodurans
Bacillus megaterium
Bacillus mycoides
Bacillus pseudomycoides
Bacillus stearothermophilus
Bacillus subtilis
Bacillus thuringiensis
Bacillus weihenstephanensis
Bacteroides distasonis
Bacteroides fragilis
Bacteroides forsythus
Bacteroides ovatus
Bacteroides vulgatus
Bartonella henselae
Bifidobacterium adolescentis
Bifidobacterium breve
Bifidobacterium dentium
Bifidobacterium longum
Blastochloris viridis
Borrelia burgdorferi
Bordetella pertussis
Bordetella bronchiseptica
Brucella abortus
Brevibacterium linens
Brevibacterium flavum
Brevundimonas diminuta
Buchnera aphidicola
Budvicia aquatica
Burkholderia cepacia
Burkholderia mallei
Burkholderia pseudomallei
Buttiauxella agrestis
Butyrivibrio fibrisolvens
Campylobacter coli
Campylobacter curvus
Campylobacter fetus subsp. *fetus*
Campylobacter fetus subsp. *venerealis*
Campylobacter gracilis
Campylobacter jejuni
Campylobacter jejuni subsp. *doylei*
Campylobacter jejuni subsp. *jejuni*
Campylobacter lari
Campylobacter rectus
Campylobacter sputorum *subsp.* sputorum
Campylobacter upsaliensis
Cedecea davisae
Cedecea lapagei
Cedecea neteri
Chlamydia pneumoniae
Chlamydia psittaci
Chlamydia trachomatis
Chlorobium vibrioforme
Chloroflexus aurantiacus
Chryseobacterium meningosepticum
Citrobacter amalonaticus
Citrobacter braakii
Citrobacter farmeri
Citrobacter freundii
Citrobacter koseri
Citrobacter sedlakii
Citrobacter werkmanii
Citrobacter youngae
Clostridium acetobutylicum
Clostridium beijerinckii
Clostridium bifermentans
Clostridium botulinum
Clostridium difficile
Clostridium innocuum
Clostridium histolyticum
Clostridium novyi
Clostridium septicum
Clostridium perfringens
Clostridium ramosum
Clostridium sordellii
Clostridium tertium
Clostridium tetani
Comamonas acidovorans
Corynebacterium accolens
Corynebacterium bovis
Corynebacterium cervicis

Table 4. **Example of microbial species for which *tuf* and/or *atpD* and/or *recA* nucleic acids and/or sequences are used in the present invention (c ntinued).**

Bacterial species (continued)

Corynebacterium diphtheriae
Corynebacterium flavescens
Corynebacterium genitalium
Corynebacterium glutamicum
Corynebacterium jeikeium
Corynebacterium kutscheri
Corynebacterium minutissimum
Corynebacterium mycetoides
Corynebacterium pseudodiphtheriticum
Corynebacterium pseudogenitalium
Corynebacterium pseudotuberculosis
Corynebacterium renale
Corynebacterium striatum
Corynebacterium ulcerans
Corynebacterium urealyticum
Corynebacterium xerosis
Coxiella burnetii
Cytophaga lytica
Deinococcus radiodurans
Deinonema sp.
Edwardsiella hoshinae
Edwardsiella tarda
Ehrlichia canis
Ehrlichia risticii
Eikenella corrodens
Enterobacter aerogenes
Enterobacter agglomerans
Enterobacter amnigenus
Enterobacter asburiae
Enterobacter cancerogenus
Enterobacter cloacae
Enterobacter gergoviae
Enterobacter hormaechei
Enterobacter sakazakii
Enterococcus avium
Enterococcus casseliflavus
Enterococcus cecorum
Enterococcus columbae
Enterococcus dispar
Enterococcus durans
Enterococcus faecalis
Enterococcus faecium
Enterococcus flavescens
Enterococcus gallinarum
Enterococcus hirae
Enterococcus malodoratus
Enterococcus mundtii
Enterococcus pseudoavium
Enterococcus raffinosus
Enterococcus saccharolyticus
Enterococcus solitarius
Enterococcus sulfureus
Erwinia amylovora
Erwinia carotovora
Escherichia coli
Escherichia fergusonii
Escherichia hermannii
Escherichia vulneris
Eubacterium lentum
Eubacterium nodatum
Ewingella americana
Francisella tularensis
Frankia alni
Fervidobacterium islandicum
Fibrobacter succinogenes
Flavobacterium ferrigeneum
Flexistipes sinusarabici
Fusobacterium gonidiaformans
Fusobacterium necrophorum subsp. *necrophorum*
Fusobacterium nucleatum subsp. *polymorphum*
Gardnerella vaginalis
Gemella haemolysans
Gemella morbillorum
Globicatella sanguis
Gloeobacter violaceus
Gloeothece sp.
Gluconobacter oxydans
Haemophilus actinomycetemcomitans
Haemophilus aphrophilus
Haemophilus ducreyi
Haemophilus haemolyticus
Haemophilus influenzae
Haemophilus parahaemolyticus
Haemophilus parainfluenzae
Haemophilus paraphrophilus
Haemophilus segnis
Hafnia alvei
Halobacterium marismortui
Halobacterium salinarum
Haloferax volcanii
Helicobacter pylori
Herpetoshiphon aurantiacus
Kingella kingae
Klebsiella ornithinolytica
Klebsiella oxytoca
Klebsiella planticola
Klebsiella pneumoniae subsp. *ozaenae*
Klebsiella pneumoniae subsp. *pneumoniae*
Klebsiella pneumoniae subsp. *rhinoscleromatis*
Klebsiella terrigena
Kluyvera ascorbata
Kluyvera cryocrescens
Kluyvera georgiana
Kocuria kristinae
Lactobacillus acidophilus
Lactobacillus garvieae
Lactobacillus paracasei
Lactobacillus casei subsp. *casei*
Lactococcus garvieae
Lactococcus lactis
Lactococcus lactis subsp. *lactis*
Leclercia adecarboxylata
Legionella micdadei

Table 4. **Example f microbial species for which *tuf* and/or *atpD* and/or *recA* nucleic acids and/or sequences are used in the present inventi n (c ntinued).**

Bacterial species (continued)

Legionella pneumophila subsp. *pneumophila*
Leminorella grimontii
Leminorella richardii
Leptospira biflexa
Leptospira interrogans
Leuconostoc mesenteroides subsp. *dextranicum*
Listeria innocua
Listeria ivanovii
Listeria monocytogenes
Listeria seeligeri
Macrococcus caseolyticus
Magnetospirillum magnetotacticum
Megamonas hypermegale
Methanobacterium thermoautotrophicum
Methanococcus jannaschii
Methanococcus vannielii
Methanosarcina barkeri
Methanosarcina jannaschii
Methylobacillus flagellatum
Methylomonas clara
Micrococcus luteus
Micrococcus lylae
Mitsuokella multacidus
Mobiluncus curtisii subsp. *holmesii*
Moellerella thermoacetica
Moellerella wisconsensis
Moorella thermoacetica
Moraxella catarrhalis
Moraxella osloensis
Morganella morganii subsp. *morganii*
Mycobacterium avium
Mycobacterium bovis
Mycobacterium gordonae
Mycobacterium kansasii
Mycobacterium leprae
Mycobacterium terrae
Mycobacterium tuberculosis
Mycoplasma capricolum
Mycoplasma gallisepticum
Mycoplasma genitalium
Mycoplasma hominis
Mycoplasma pirum
Mycoplasma mycoides
Mycoplasma pneumoniae
Mycoplasma pulmonis
Mycoplasma salivarium
Myxococcus xanthus
Neisseria animalis
Neisseria canis
Neisseria cinerea
Neisseria cuniculi
Neisseria elongata subsp. *elongata*
Neisseria elongata subsp. *intermedia*
Neisseria flava
Neisseria flavescens
Neisseria gonorrhoeae
Neisseria lactamica
Neisseria meningitidis
Neisseria mucosa
Neisseria perflava
Neisseria pharyngis var. *flava*
Neisseria polysaccharea
Neisseria sicca
Neisseria subflava
Neisseria weaveri
Obesumbacterium proteus
Ochrobactrum anthropi
Pantoea agglomerans
Pantoea dispersa
Paracoccus denitrificans
Pasteurella multocida
Pectinatus frisingensis
Peptococcus niger
Peptostreptococcus anaerobius
Peptostreptococcus asaccharolyticus
Peptostreptococcus prevotii
Phormidium ectocarpi
Pirellula marina
Planobispora rosea
Plesiomonas shigelloides
Plectonema boryanum
Porphyromonas asaccharolytica
Porphyromonas gingivalis
Pragia fontium
Prevotella buccalis
Prevotella melaninogenica
Prevotella oralis
Prevotella ruminocola
Prochlorothrix hollandica
Propionibacterium acnes
Propionigenium modestum
Proteus mirabilis
Proteus penneri
Proteus vulgaris
Providencia alcalifaciens
Providencia rettgeri
Providencia rustigianii
Providencia stuartii
Pseudomonas aeruginosa
Pseudomonas fluorescens
Pseudomonas putida
Pseudomonas stutzeri
Psychrobacter phenylpyruvicum
Pyrococcus abyssi
Rahnella aquatilis
Rickettsia prowazekii
Rhizobium leguminosarum
Rhizobium phaseoli
Rhodobacter capsulatus
Rhodobacter sphaeroides

Table 4. Example of microbial species f r which *tuf* and/or *atpD* and/or *recA* nucleic acids and/or sequences are used in the present invention (continued).

Bacterial species (continued)

Rhodopseudomonas palustris
Rhodospirillum rubrum
Ruminococcus albus
Ruminococcus bromii
Salmonella bongori
Salmonella choleraesuis subsp. *arizonae*
Salmonella choleraesuis subsp *choleraesuis*
Salmonella choleraesuis subsp. *diarizonae*
Salmonella choleraesuis subsp. *houtenae*
Salmonella choleraesuis subsp. *indica*
Salmonella choleraesuis subsp. *salamae*
Serpulina hyodysenteriae
Serratia ficaria
Serratia fonticola
Serratia grimesii
Serratia liquefaciens
Serratia marcescens
Serratia odorifera
Serratia plymuthica
Serratia rubidaea
Shewanella putrefaciens
Shigella boydii
Shigella dysenteriae
Shigella flexneri
Shigella sonnei
Sinorhizobium meliloti
Spirochaeta aurantia
Staphylococcus aureus
Staphylococcus aureus subsp. *aureus*
Staphylococcus auricularis
Staphylococcus capitis subsp. *capitis*
Staphylococcus cohnii subsp. *cohnii*
Staphylococcus epidermidis
Staphylococcus haemolyticus
Staphylococcus hominis
Staphylococcus hominis subsp. *hominis*
Staphylococcus lugdunensis
Staphylococcus saprophyticus
Staphylococcus sciuri subsp. *sciuri*
Staphylococcus simulans
Staphylococcus warneri
Stigmatella aurantiaca
Stenotrophomonas maltophilia
Streptococcus acidominimus
Streptococcus agalactiae
Streptococcus anginosus
Streptococcus bovis
Streptococcus cricetus
Streptococcus cristatus
Streptococcus downei
Streptococcus dysgalactiae
Streptococcus equi subsp. *equi*
Streptococcus ferus
Streptococcus gordonii
Streptococcus macacae
Streptococcus mitis
Streptococcus mutans
Streptococcus oralis
Streptococcus parasanguinis
Streptococcus pneumoniae
Streptococcus pyogenes
Streptococcus ratti
Streptococcus salivarius
Streptococcus salivarius subsp. *thermophilus*
Streptococcus sanguinis
Streptococcus sobrinus
Streptococcus suis
Streptococcus uberis
Streptococcus vestibularis
Streptomyces anbofaciens
Streptomyces aureofaciens
Streptomyces cinnamoneus
Streptomyces coelicolor
Streptomyces collinus
Streptomyces lividans
Streptomyces netropsis
Streptomyces ramocissimus
Streptomyces rimosus
Streptomyces venezuelae
Succinivibrio dextrinosolvens
Synechococcus sp.
Synechocystis sp.
Tatumella ptyseos
Taxeobacter occealus
Tetragenococcus halophilus
Thermoplasma acidophilum
Thermotoga maritima
Thermus aquaticus
Thermus thermophilus
Thiobacillus ferrooxidans
Thiomonas cuprina
Trabulsiella guamensis
Treponema pallidum
Ureaplasma urealyticum
Veillonella parvula
Vibrio alginolyticus
Vibrio anguillarum
Vibrio cholerae
Vibrio mimicus
Wolinella succinogenes
Xanthomonas citri
Xanthomonas oryzae
Xenorhabdus bovieni
Xenorhabdus nematophilus
Yersinia bercovieri
Yersinia enterocolitica
Yersinia frederiksensii
Yersinia intermedia
Yersinia pestis

Table 4. Example of microbial species f r which *tuf* and/or *atpD* and/or *recA* nucleic acids and/or sequences are used in the present invention (continued).

Bacterial species (continued)

Yersinia pseudotuberculosis
Yersinia rohdei
Yokenella regensburgei
Zoogloea ramigera

Table 4. Example of micr bial species f r which *tuf* and/or *atpD* and/ r *recA* nucleic acids and/or sequences are used in the present inventi n (c ntinued).

Fungal species

Absidia corymbifera
Absidia glauca
Alternaria alternata
Arxula adeninivorans
Aspergillus flavus
Aspergillus fumigatus
Aspergillus nidulans
Aspergillus niger
Aspergillus oryzae
Aspergillus terreus
Aspergillus versicolor
Aureobasidium pullulans
Basidiobolus ranarum
Bipolaris hawaiiensis
Bilophila wadsworthia
Blastoschizomyces capitatus
Blastomyces dermatitidis
Candida albicans
Candida catenulata
Candida dubliniensis
Candida famata
Candida glabrata
Candida guilliermondii
Candida haemulonii
Candida inconspicua
Candida kefyr
Candida krusei
Candida lambica
Candida lusitaniae
Candida norvegica
Candida norvegensis
Candida parapsilosis
Candida rugosa
Candida sphaerica
Candida tropicalis
Candida utilis
Candida viswanathii
Candida zeylanoides
Cladophialophora carrionii
Coccidioides immitis
Coprinus cinereus
Cryptococcus albidus
Cryptococcus humicolus
Cryptococcus laurentii
Cryptococcus neoformans
Cunninghamella bertholletiae
Curvularia lunata
Emericella nidulans
Emmonsia parva
Eremothecium gossypii
Exophiala dermatitidis
Exophiala jeanselmei
Exophiala moniliae
Exserohilum rostratum
Eremothecium gossypii
Fonsecaea pedrosoi
Fusarium moniliforme
Fusarium oxysporum
Fusarium solani
Geotrichum sp.
Histoplasma capsulatum
Hortaea werneckii
Issatchenkia orientalis Kudrjanzev
Kluyveromyces lactis
Malassezia furfur
Malassezia pachydermatis
Malbranchea filamentosa
Metschnikowia pulcherrima
Microsporum audouinii
Microsporum canis
Mucor circinelloides
Neurospora crassa
Paecilomyces lilacinus
Paracoccidioides brasiliensis
Penicillium marneffei
Phialaphora verrucosa
Pichia anomala
Piedraia hortai
Podospora anserina
Podospora curvicolla
Puccinia graminis
Pseudallescheria boydii
Reclinomonas americana
Rhizomucor racemosus
Rhizopus oryzae
Rhodotorula minuta
Rhodotorula mucilaginosa
Saccharomyces cerevisiae
Saksenaea vasiformis
Schizosaccharomyces pombe
Scopulariopsis koningii
Sordaria macrospora
Sporobolomyces salmonicolor
Sporothrix schenckii
Stephanoascus ciferrii
Syncephalastrum racemosum
Trichoderma reesei
Trichophyton mentagrophytes
Trichophyton rubrum
Trichophyton tonsurans
Trichosporon cutaneum
Ustilago maydis
Wangiella dermatitidis
Yarrowia lipolytica

Table 4. Example of micr bial species f r which *tuf* and/ r *atpD* and/or *recA* nucleic acids and/or sequences are used in the present invention (continued).

Parasitical species
Babesia bigemina
Babesia bovis
Babesia microti
Blastocystis hominis
Crithidia fasciculata
Cryptosporidium parvum
Entamoeba histolytica
Giardia lamblia
Kentrophoros sp.
Leishmania aethiopica
Leishmania amazonensis
Leishmania braziliensis
Leishmania donovani
Leishmania infantum
Leishmania enriettii
Leishmania gerbilli
Leishmania guyanensis
Leishmania hertigi
Leishmania major
Leishmania mexicana
Leishmania panamensis
Leishmania tarentolae
Leishmania tropica
Neospora caninum
Onchocerca volvulus
Plasmodium berghei
Plasmodium falciparum
Plasmodium knowlesi
Porphyra purpurea
Toxoplasma gondii
Treponema pallidum
Trichomonas tenax
Trichomonas vaginalis
Trypanosoma brucei
Trypanosoma brucei subsp. *brucei*
Trypanosoma congolense
Trypanosoma cruzi

Table 5. Antimicrobial agents resistance genes selected for diagn stic purposes.

Gene	Antimicrobial agent	Bacteria[1]	ACCESSION NO.	SEQ ID NO.
aac(3)-Ib [2]	Aminoglycosides	*Enterobacteriaceae* *Pseudomonads*	L06157	
aac(3)-IIb [2]	Aminoglycosides	*Enterobacteriaceae*, Pseudomonads	M97172	
aac(3)-IVa [2]	Aminoglycosides	*Enterobacteriaceae*	X01385	
aac(3)-VIa [2]	Aminoglycosides	*Enterobacteriaceae*, Pseudomonads	M88012	
aac(2')-Ia [2]	Aminoglycosides	*Enterobacteriaceae*, Pseudomonads	X04555	
aac(6')-aph(2'') [2]	Aminoglycosides	*Enterococcus* sp., *Staphylococcus* sp.		83-86 [3]
aac(6')-Ia, [2]	Aminoglycosides	*Enterobacteriaceae*, Pseudomonads	M18967	
aac(6')-Ic [2]	Aminoglycosides	*Enterobacteriaceae*, Pseudomonads	M94066	
aac(6')-IIa [2]	Aminoglycosides	Pseudomonads		112 [4]
aadB [*ant(2")-Ia* [2]]	Aminoglycosides	*Enterobacteriaceae*		53-54 [3]
aacC1 [*aac(3)-Ia* [2]]	Aminoglycosides	Pseudomonads		55-56 [3]
aacC2 [*aac(3)-IIa* [2]]	Aminoglycosides	Pseudomonads		57-58 [3]
aacC3 [*aac(3)-III* [2]]	Aminoglycosides	Pseudomonads		59-60 [3]
aacA4 [*aac(6')-Ib* [2]]	Aminoglycosides	Pseudomonads		65-66 [3]
ant(3")-Ia [2]	Aminoglycosides	*Enterobacteriaceae*, *Enterococcus* sp., *Staphylococcus* sp.	X02340 M10241	
ant(4')-Ia [2]	Aminoglycosides	*Staphylococcus* sp.	V01282	
aph(3')-Ia [2]	Aminoglycosides	*Enterobacteriaceae*, Pseudomonads	J01839	
aph(3')-IIa [2]	Aminoglycosides	*Enterobacteriaceae*, Pseudomonads	V00618	
aph(3')-IIIa [2]	Aminoglycosides	*Enterococcus* sp., *Staphylococcus* sp.	V01547	
aph(3')-VIa [2]	Aminoglycosides	*Enterobacteriaceae*, Pseudomonads	X07753	
rpsL [2]	Streptomycin	*M. tuberculosis*, *M. avium complex*	X80120 U14749 X70995 L08011	
bla_{OXA} [5,6]	ß-lactams	*Enterobacteriaceae*, Pseudomonads	Y10693 AJ238349 AJ009819 X06046 X03037 X07260 U13880 X75562 AF034958 J03427 Z22590 U59183 L38523 U63835 AF043100 AF060206 U85514 AF043381 AF024602 AF064820	110 [4]
bla_{ROB} [5]	ß-lactams	*Haemophilus* sp. *Pasteurella* sp.		45-48 [3]

Table 5. Antimicr bial agents resistance genes selected for diagnostic purposes (c ntinued).

Gene	Antimicrobial agent	Bacteria[1]	ACCESSION NO.	SEQ ID NO.
bla$_{SHV}$ [5,6]	ß-lactams	*Enterobacteriacea,*	AF124984	41-44 [3]
		Pseudomonas aeruginosa	AF148850	
			M59181	
			X98099	
			M33655	
			AF148851	
			X53433	
			L47119	
			AF074954	
			X53817	
			AF096930	
			X55640	
			Y11069	
			U20270	
			U92041	
			S82452	
			X98101	
			X98105	
			AF164577	
			AJ011428	
			AF116855	
			AB023477	
			AF293345	
			AF227204	
			AF208796	
			AF132290	
bla$_{TEM}$ [5,6]	ß-lactams	*Enterobacteriaceae,*	AF012911	37-40 [3]
		Neisseria sp.,	U48775	
		Haemophilus sp.	AF093512	
			AF052748	
			X64523	
			Y13612	
			X57972	
			AF157413	
			U31280	
			U36911	
			U48775	
			V00613	
			X97254	
			AJ012256	
			X04515	
			AF126482	
			U09188	
			M88143	
			Y14574	
			AF188200	
			AJ251946	
			Y17581	
			Y17582	
			Y17583	
			M88143	
			U37195	
			Y17584	
			X64523	
			U95363	
			Y10279	
			Y10280	
			Y10281	
			AF027199	
			AF104441	
			AF104442	
			AF062386	
			X57972	
			AF047171	
			AF188199	
			AF157553	
			AF190694	
			AF190695	
			AF190693	
			AF190692	

Table 5. Antimicr bial agents resistance genes selected for diagn stic purposes (c ntinued).

Gene	Antimicrobial agent	Bacteria[1]	ACCESSION NO.	SEQ ID NO.
bla_{CARB} [5]	ß-lactams	*Pseudomonas* sp.,	J05162	
		Enterobacteriaceae	S46063	
			M69058	
			U14749	
			D86225	
			D13210	
			Z18955	
			AF071555	
			AF153200	
			AF030945	
$bla_{CTX\text{-}M\text{-}1}$ [5]	ß-lactams	*Enterobacteriaceae*	X92506	
$bla_{CTX\text{-}M\text{-}2}$ [5]	ß-lactams	*Enterobacteriaceae*	X92507	
$bla_{CMY\text{-}2}$ [7]	ß-lactams	*Enterobacteriaceae*	X91840	
			AJ007826	
			AJ011293	
			AJ011291	
			Y17716	
			Y16783	
			Y16781	
			Y15130	
			U77414	
			S83226	
			Y15412	
			X78117	
bla_{IMP} [5]	ß-lactams	*Enterobacteriaceae*,	AJ223604	
		Pseudomonas aeruginosa	S71932	
			D50438	
			D29636	
			X98393	
			AB010417	
			D78375	
$bla_{PER\text{-}1}$ [5]	ß-lactams	*Enterobacteriaceae*, *Pseudomodanaceae*	Z21957	
$bla_{PER\text{-}2}$ [7]	ß-lactams	*Enterobacteriaceae*	X93314	
blaZ[12]	ß-lactams	*Enterococcus* sp., *Staphylococcus* sp.		111 [4]
mecA[12]	ß-lactams	*Staphylococcus* sp.		97-98 [3]

Table 5. Antimicr bial agents resistance genes selected for diagnostic purp ses (c ntinued).

Gene	Antimicrobial agent	Bacteria[1]	ACCESSION NO.	SEQ ID NO.
pbp1a[13]	ß-lactams	*Streptococcus pneumoniae*	M90527	1004-1018, 1648,2056-2064, 2273-2276
			X67872	
			AB006868	
			AB006874	
			X67873	
			AB006878	
			AB006875	
			AB006877	
			AB006879	
			AF046237	
			AF046235	
			AF026431	
			AF046232	
			AF046233	
			AF046236	
			X67871	
			Z49095	
			AF046234	
			AB006873	
			X67866	
			X67868	
			AB006870	
			AB006869	
			AB006872	
			X67870	
			AB006871	
			X67867	
			X67869	
			AB006876	
			AF046230	
			AF046238	
			Z49094	
pbp2b[13]	ß-lactams	*Streptococcus pneumoniae*	X16022	1019-1033
			M25516	
			M25518	
			M25515	
			U20071	
			U20084	
			U20082	
			U20067	
			U20079	
			Z22185	
			U20072	
pbp2b[13]	ß-lactams	*Streptococcus pneumoniae*	U20083	
			U20081	
			M25522	
			U20075	
			U20070	
			U20077	
			U20068	
			Z22184	
			U20069	
			U20078	
			M25521	
			M25525	
			M25519	
			Z21981	
			M25523	
			M25526	
			U20076	
			U20074	
			M25520	
			M25517	
			M25524	
			Z22230	
			U20073	
			U20080	

Table 5. Antimicr bial agents resistance genes selected f r diagn stic purposes (c ntinued).

Gene	Antimicrobial agent	Bacteria[1]	ACCESSION NO.	SEQ ID NO.
pbp2x [13]	ß-lactams	*Streptococcus pneumoniae*	X16367 X65135 AB011204 AB011209 AB011199 AB011200 AB011201 AB011202 AB011198 AB011208 AB011205 AB015852 AB011210 AB015849 AB015850 AB015851 AB015847 AB015846 AB011207 AB015848 Z49096	1034-1048
int	-lactams, trimethoprim	*Enterobacteriaceae*,		99-102 [3]
sul	aminoglycosides, antiseptic, chloramphenicol	Pseudomonads		103-106 [3]
ermA [14]	Macrolides, lincosamides, streptogramin B	*Staphylococcus* sp.		113 [4]
ermB [14]	Macrolides, lincosamides, streptogramin B	*Enterobacteriaceae*, *Staphylococcus* sp. *Enterococcus* sp. *Streptococcus* sp.		114 [4]
ermC [14]	Macrolides, lincosamides, streptogramin B	*Enterobacteriaceae*, *Staphylococcus* sp.		115 [4]
ereA [12]	Macrolides	*Enterobacteriaceae*, *Staphylococcus* sp.	M11277 E01199 AF099140	
ereB [12]	Macrolides	*Enterobacteriaceae* *Staphylococcus* sp.	A15097 X03988	
msrA [12]	Macrolides	*Staphylococcus* sp.		77-80 [3]
mefA, mefE [8]	Macrolides	*Streptococcus* sp.	U70055 U83667	
mphA [8]	Macrolides	*Enterobacteriaceae*, *Staphylococcus* sp.	D16251 U34344 U36578	
linA/linA' [9]	Lincosamides	*Staphylococcus* sp.	J03947 M14039 A15070 E01245	
linB [10]	Lincosamides	*Enterococcus faecium*	AF110130 AJ238249	
vga [15]	Streptrogramin	*Staphylococcus* sp.	M90056 U82085	89-90 [3]
vgb [15]	Streptrogramin	*Staphylococcus* sp.	M36022 M20219 AF015628	

Table 5. Antimicrobial agents resistance genes selected for diagnostic purposes (continued).

Gene	Antimicrobial agent	Bacteria[1]	ACCESSION NO.	SEQ ID NO.
vat [15]	Streptrogramin	*Staphylococcus* sp.	L07778	87-88 [3]
vatB [15]	Streptrogramin	*Staphylococcus* sp.	U19459 L38809	
satA [15]	Streptrogramin	*Enterococcus faecium*	L12033	81-82 [3]
mupA [12]	Mupirocin	*Staphylococcus aureus*	X75439 X59478 X59477	
gyrA [16]	Quinolones	Gram-positive and gram-negative bacteria	X95718 X06744 X57174 X16817 X71437 AF065152 AF060881 D32252	1255, 1607-1608, 1764-1776, 2013-2014, 2277-2280
parC/grlA [16]	Quinolones	Gram-positive and gram-negative bacteria	AB005036 AF056287 X95717 AF129764 AB017811 AF065152	1777-1785
parE/grlB [16]	Quinolones	Gram-positive bacteria	X95717 AF065153 AF058920	
norA [16]	Quinolones	*Staphylococcus* sp.	D90119 M80252 M97169	
mexR (nalB) [16]	Quinolones	*Pseudomonas aeruginosa*	U23763	
nfxB [16]	Quinolones	*Pseudomonas aeruginosa*	X65646	
cat [12]	Chloramphenicol	Gram-positive and gram-negative bacteria	M55620 X15100 A24651 M28717 A00568 A00569 X74948 Y00723 A24362 A00569 M93113 M62822 M58516 V01277 X02166 M77169 X53796 J01841 X07848	
ppflo-like	Chloramphenicol		AF071555	
embB [17]	Ethambutol	*Mycobacterium tuberculosis*	U68480	
pncA [17]	Pyrazinamide	*Mycobacterium tuberculosis*	U59967	
rpoB [17]	Rifampin	*Mycobacterium tuberculosis*	AF055891 AF055892 S71246 L27989 AF055893	
inhA [17]	Isoniazid	*Mycobacterium tuberculosis*	AF106077 U02492	

Table 5. Antimicrobial agents resistance genes selected f r diagn stic purp ses (c ntinued).

Gene	Antimicrobial agent	Bacteria[1]	ACCESSION NO.	SEQ ID NO.
vanA [12]	Vancomycin	*Enterococcus* sp.		67-70[3]
				1049-1057
vanB [12]	Vancomycin	*Enterococcus* sp.		116[4]
vanC1 [12]	Vancomycin	*Enterococcus gallinarum*		117[4]
				1058-1059
vanC2 [12]	Vancomycin	*Enterococcus casseliflavus*		1060-1063
			U94521	
			U94522	
			U94523	
			U94524	
			U94525	
			L29638	
vanC3 [12]	Vancomycin	*Enterococcus flavescens*		1064-1066
			L29639	
			U72706	
vanD [18]	Vancomycin	*Enterococcus faecium*	AF130997	
vanE [12]	Vancomycin	*Enterococcus faecium*	AF136925	
tetB [19]	Tetracycline	Gram-negative bacteria	J01830	
			AF162223	
			AP000342	
			S83213	
			U81141	
			V00611	
tetM [19]	Tetracycline	Gram-negative and	X52632	
		Gram-positive bacteria	AF116348	
			U50983	
			X92947	
			M211136	
			U08812	
			X04388	
sul II [20]	Sulfonamides	Gram-negative bacteria	M36657	
			AF017389	
			AF017391	
dhfrIa [20]	Trimethoprim	Gram-negative bacteria	AJ238350	
			X17477	
			K00052	
			U09476	
			X00926	
dhfrIb [20]	Trimethoprim	Gram-negative bacteria	Z50805	
			Z50804	
dhfrV [20]	Trimethoprim	Gram-negative bacteria	X12868	
dhfrVI [20]	Trimethoprim	Gram-negative bacteria	Z86002	
dhfrVII [20]	Trimethoprim	Gram-negative bacteria	U31119	
			AF139109	
			X58425	
dhfrVIII [20]	Trimethoprim	Gram-negative bacteria	U10186	
			U09273	
dhfrIX [20]	Trimethoprim	Gram-negative bacteria	X57730	
dhfrXII [20]	Trimethoprim	Gram-negative bacteria	Z21672	
			AF175203	
			AF180731	
			M84522	
dhfrXIII [20]	Trimethoprim	Gram-negative bacteria	Z50802	
dhfrXV [20]	Trimethoprim	Gram-negative bacteria	Z83331	
dhfrXVII [20]	Trimethoprim	Gram-negative bacteria	AF170088	
			AF180469	
			AF169041	

Table 5. Antimicrobial agents resistanc genes selected for diagn stic purposes (continued).

Gene	Antimicrobial agent	Bacteria[1]	ACCESSION NO.	SEQ ID NO.
dfrA [20]	Trimethoprim	*Staphylococcus* sp.	AF045472 U40259 AF051916 X13290 Y07536 Z16422 Z48233	

1 Bacteria having high incidence for the specified antibiotic resistance gene. The presence of the antibiotic resistance genes in other bacteria is not excluded.

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3 Antibiotic resistance genes from our assigned US patent no. 6,001,564 for which we have selected PCR primer pairs.

4 These SEQ ID NOs. refer to a previous patent (publication WO98/20157).

5 Bush, K., G.A. Jacoby and A. Medeiros. 1995. A functional classification scheme for ß-lactamase and its correlation with molecular structure. Antimicrob. Agents. Chemother. 39:1211-1233.

6 Nucleotide mutations in bla_{SHV}, bla_{TEM}, and bla_{OXA}, are associated with extended-spectrum ß-lactamase or inhibitor-resistant ß-lactamase.

7 Bauerfeind, A., Y. Chong, and K. Lee. 1998. Plasmid-encoded AmpC beta-lactamases: how far have we gone 10 ears after discovery? Yonsei Med. J. 39:520-525.

8 Sutcliffe, J., T. Grebe, A. Tait-Kamradt, and L. Wondrack. 1996. Detection of erythromycin-resistant determinants by PCR. Antimicrob. Agent Chemother. 40:2562-2566.

9 Leclerc, R., A., Brisson-Noël, J. Duval, and P. Courvalin. 1991. Phenotypic expression and genetic heterogeneity of lincosamide inactivation in Staphylococcus sp. Antimicrob. Agents. Chemother. 31:1887-1891.

10 Bozdogan, B., L. Berrezouga, M.-S. Kuo, D. A. Yurek, K. A. Farley, B. J. Stockman, and R. Leclercq. 1999. A new gene, linB, conferring resistance to lincosamides by nucleotidylation in Enterococcus faecium HM1025. Antimicrob. Agents. Chemother. 43:925-929.

11 Cockerill III, F.R. 1999. Genetic methods for assessing antimicrobial resistance. Antimicrob. Agents. Chemother. 43:199-212.

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13 Dowson, C. G., T. J. Tracey, and B. G. Spratt. 1994. Origin and molecular epidemiology of penicillin-binding-protein-mediated resistance to ß-lactam antibiotics. Trends Molec. Microbiol.2: 361-366.

14 Jensen, L. B., N. Frimodt-Moller, F. M. Aarestrup. 1999. Presence of erm gene classes in Gram-positive bacteria of animal and human origin in Denmark. FEMS Microbiol. 170:151-158.

15 Thal, L. A., and M. J. Zervos. 1999. Occurrence and epidemiology of resistance to virginimycin and streptrogramins. J. Antimicrob. Chemother. 43:171-176.

16 Martinez J. L., A. Alonso, J. M. Gomez-Gomez, and F. Baquero. 1998. Quinolone resistance by mutations in chromosomal gyrase genes. Just the tip of the iceberg? J. Antimicrob. Chemother. 42:683-688

17 Cockerill III, F.R. 1999. Genetic methods for assessing antimicrobial resistance. Antimicrob. Agents. Chemother. 43:199-212.

18 Casadewall, B. and P. Courvalin. 1999 Characterization of the vanD glycopeptide resistance gene cluster from Enterococcus faecium BM 4339. J. Bacteriol. 181:3644-3648.

19 Roberts, M.C. 1999. Genetic mobility and distribution of tetracycline resistance determinants.Ciba Found. Symp. 207:206-222.

20 Huovinen, P., L. Sundström, G. Swedberg, and O. Sköld. 1995. Trimethoprim and sulfonamide resistance.Antimicrob. Agent Chemother. 39:279-289.

Table 6. List of bacterial t xins select d for diagn stic purposes.

Organism	Toxin	Accessi n number
Actinobacillus actinomycetemcomitans	Cytolethal distending toxin (*cdt*A, *cdt*B, *cdt*C)	AF006830
	Leukotoxin (*ltx*A)	M27399
Actinomyces pyogenes	Hemolysin (pyolysin)	U84782
Aeromonas hydrophila	Aerolysin (aerA)	M16495
	Haemolysin (hlyA)	U81555
	Cytotonic enterotoxin (*alt*)	L77573
Bacillus anthracis	Anthrax toxin (*cya*)	M23179
Bacillus cereus	Enterotoxin (*bce*T)	D17312 AF192766, AF192767
	Enterotoxic hemolysin BL	AJ237785
	Non-haemolytic enterotoxins A,B and C (*nhe*)	Y19005
Bacillus mycoides	Hemolytic enterotoxin HBL	AJ243150 to AJ243153
Bacillus pseudomycoides	Hemolytic enterotoxin HBL	AJ243154 to AJ243156
Bacteroides fragilis	Enterotoxin (*bft*P)	U67735
	Matrix metalloprotease/enterotoxin (fragilysin)	S75941, AF038459
	Metalloprotease toxin-2	U90931 AF081785
	Metalloprotease toxin-3	AF056297
Bordetella bronchiseptica	Adenylate cyclase hemolysin (*cya*A)	Z37112, U22953
	Dermonecrotic toxin (*dnt*)	U59687 AB020025
Bordetella pertussis	Pertussis toxin (S1 subunit, *tox*)	AJ006151 AJ006153 AJ006155 AJ006157 AJ006159 AJ007363 M14378, M16494 AJ007364 M13223 X16347
	Adenyl cyclase (*cya*)	18323
	Dermonecrotic toxin (*dnt*)	U10527
Campylobacter jejuni	Cytolethal distending toxin (*cdt*A, *cdt*B, *cdt*C)	U51121
Citrobacter freundii	Shiga-like toxin (*slt*-IIcA)	X67514, S53206
Clostridium botulinum	Botulism toxin (BoNT) (A,B,E and F serotypes are neurotoxic for humans; the other serotypes have not been considered)	X52066, X52088 X73423 M30196 X70814 X70819 X71343 Z11934 X70817 M81186 X70818 X70815 X62089 X62683 S76749 X81714 X70816

Table 6. List of bacterial toxins selected f r diagn stic purposes (continued).

Organism	Toxin	Accession number
Clostridium botulinum (continued)		X70820 X70281 L35496 M92906
Clostridium difficile	A toxin (enterotoxin) (*tcd*A) (*cdtA*)	AB012304 AF053400 Y12616 X51797 X17194 M30307
	B toxin (cytotoxin) (*tox*B) (*cdtB*)	Z23277 X53138
Clostridium perfringens	Alpha (phospholipase C) (*cpa*)	L43545 L43546 L43547 L43548 X13608 X17300 D10248
	Beta (dermonecrotic protein) (*cpb*)	L13198 X83275 L77965
	Enterotoxin (*cpe*)	AJ000766 M98037 X81849 X71844 Y16009
	Enterotoxin pseudogene (not expressed)	AF037328 AF037329 AF037330
	Epsilon toxin (*etx*D)	M80837 M95206 X60694
	Iota (Ia and Ib)	X73562
	Lambda (metalloprotease)	D45904
	Theta (perfringolysin O)	M36704
Clostridium sordellii	Cytotoxin L	X82638
Clostridium tetani	Tetanos toxin	X06214 X04436
Corynebacterium diphtheriae	Diphtheriae toxin	X00703
Corynebacterium pseudotuberculosis	Phospholipase C	A21336
Eikenella corrodens	lysine decarboxylase (*cad*A)	U89166
Enterobacter cloacae	Shiga-like toxin II	Z50754, U33502
Enterococcus faecalis	Cytolysin B (*cylB*)	M38052
Escherichia coli (EHEC)	Hemolysin toxin (*hly*A and *ehx*A)	AF043471 X94129 X79839 X86087 AB011549 AF074613

Table 6. List f bacterial toxins selected for diagnostic purp ses (continued).

Organism	T xin	Accessi n number
Escherichia coli (EHEC)	Shiga-like (Vero cytotoxin) (*stx*)	X81418, M36727
		M14107, E03962
		M10133, E03959
		M12863, X07865
		X81417, Y10775
		X81416, Z50754
		X81415, X67515
		Z36900, AF043627
		L11078, M19473
		L04539, M17358
		L11079, M19437
		X65949, M24352
		M21534, X07903
		M29153, Z36899
		Z37725
		Z36901
		X61283
		AB017524
		U72191
		X61283
Escherichia coli (ETEC)	Enterotoxin (heat-labile) (*elt*B)	M17874
		M17873
		J01605
		AB011677
	Enterotoxin (heat-stable) (*ast*A) (*est*A1)	L11241
		M58746
		M29255
		V00612
		J01831
Escherichia coli *(other)*	*Cytolethal-distending toxin* (*cdt*) (3 genes)	*U03293*
		U04208
		U89305
	Cytotoxic necrotizing factor 1 (*cnf*1)	U42629
	Microcin 24 (*mtf*S)	U47048
	Autotransporter enterotoxin (*Pet*) (cytotoxin)	AF056581
Haemophilus ducreyi	Cytolethal distending toxin (*cdt*A, *cdt*B, *cdt*C)	U53215
Helicobacter pylori	Vacuolating toxin (*vac*A)	U07145
		U80067
		U80068
		AF077938
		AF077939
		AF077940
		AF077941
Legionella pneumophila	Structural toxin protein (*rtx*A)	AF057703
Listeria monocytogenes	Listeriolysin O (*lis*A, *hly*A)	X15127
		M24199
		X60035
		U25452
		U25443
		U25446
		U25449
Pasteurella multocida	Mitogenic toxin (dermonecrotic toxin)	X57775, Z28388
		X51512
		X52478
Proteus mirabilis	Hemolysin (hpmA)	M30186
Pseudomonas aeruginosa	Cytotoxin (Enterotoxin A)	X14956
Salmonella typhimurium	Calmodulin-sensitive adenylate cyalase toxin (*cya*)	AF060869
	Cytolysin (salmolysin) (*sly*A)	U03842
	Enterotoxin (*stn*)	L16014

Table 6. List of bacterial toxins selected for diagn stic purposes (continued).

Organism	T xin	Accession number
Serratia marcescens	Hemolysin (*shl*A)	M22618
Shigella dysenteriae type 1	Shiga toxin (*stx*A and *stx*B)	X07903, M32511 M19437 M24352, M21947
Shigella flexneri	ShET2 enterotoxin (*sen*A)	Z54211 Z47381
	Enterotoxin 1 (*set*1A and *set*1B)	U35656
	Hemolysin E (*hly*E, *cly*A, *she*A)	AF200955
Shigella sonnei	Shiga toxin (*stx*A and *stx*B)	AJ132761
Sphingomonas paucimobilis	Beta-hemolysin (hlyA)	L01270
Staphylococcus aureus	Gamma-hemolysin (*hlg*2)	D42143 L01055
	Enterotoxin	U93688
	Enterotoxin A (*sea*)	L22565, L22566 M18970
	Enterotoxin B	M11118
	Enterotoxin C1 (*ent*C1)	X05815
	Enterotoxin C2 (*ent*C2)	P34071
	Enterotoxin C3 (*ent*C3)	X51661
	Enterotoxin D (*sed*)	M94872
	Enterotoxin E	M21319
	Enterotoxin G (*seg*)	AF064773
	Enterotoxin H (*seh*)	U11702
	Enterotoxin I (*sei*)	AF064774
	Enterotoxin J	AF053140
	Exfoliative toxin A (ETA, Epidermolytic toxin A)	M17347 M17357 L25372, M20371
	Exfoliative toxin B (ETB)	M17348, M13775
	Leukocidin R (F and S component, *luk*F and *luk*S; Hemolysin B and C)	X64389, S53213 X72700 L01055
	Toxic shock syndrome toxin 1 (TSST-1, alpha toxin, alpha hemolysin)	X01645 M90536 J02615 U93688
Staphylococcus epidermidis	Delta toxin (*hld*)	AF068634
Staphylococcus intermedius	Enterotoxin 1	U91526
	Leukocidin R (F and S component, *luk*F and *luk*S; synergohymenotropic toxin)	X79188
Streptococcus pneumoniae	Pneumolysin	X52474

Table 6. List f bacterial t xins selected f r diagn stic purposes (c ntinued).

Organism	Toxin	Accessi n number
Streptococcus pyogenes	*Streptococcus* pyrogenic exotoxin A (*spe*A)	X61553 to X61573 X03929 U40453, M19350
	Pyrogenic exotoxin B (*spe*B) M86905, M35110	U63134
Vibrio cholerae	Cholerae toxin (*ctx*A and *ctx*B subunits)	X00171 X76390 X58786 X58785, S55782 D30052 D30053 K02679 AF175708
	Accessory cholera enterotoxin (*ace*)	Z22569, AF175708
	Heat-stable enterotoxin (*sto*)	X74108, M85198 M97591, L03220
	Zonula occludens toxin (*zot*)	M83563, AF175708
Vibrio parahaemolyticus	Thermostable direct hemolysin (*tdh*)	S67841
Vibrio vulnificus	Cytolysin (*vvh*A)	M34670
Yersinia enterocolitica	Heat-stable enterotoxin (*yst*)	U09235, X65999
	Heat-stable enterotoxin type B (*yst*B)	D88145
	Heat-stable enterotoxin type C (*yst*C)	D63578
Yersinia kristensenii	Enterotoxin X69218	
Yersinia pestis	Toxin	X92727

Table 7. Origin f the nucleic acids and/ r sequences in the sequence listing.

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
1	*Acinetobacter baumannii*	This patent	*tuf*
2	*Actinomyces meyeri*	This patent	*tuf*
3	*Aerococcus viridans*	This patent	*tuf*
4	*Achromobacter xylosoxidans* subsp. *denitrificans*	This patent	*tuf*
5	*Anaerorhabdus furcosus*	This patent	*tuf*
6	*Bacillus anthracis*	This patent	*tuf*
7	*Bacillus cereus*	This patent	*tuf*
8	*Bacteroides distasonis*	This patent	*tuf*
9	*Enterococcus casseliflavus*	This patent	*tuf*
10	*Staphylococcus saprophyticus*	This patent	*tuf*
11	*Bacteroides ovatus*	This patent	*tuf*
12	*Bartonella henselae*	This patent	*tuf*
13	*Bifidobacterium adolescentis*	This patent	*tuf*
14	*Bifidobacterium dentium*	This patent	*tuf*
15	*Brucella abortus*	This patent	*tuf*
16	*Burkholderia cepacia*	This patent	*tuf*
17	*Cedecea davisae*	This patent	*tuf*
18	*Cedecea neteri*	This patent	*tuf*
19	*Cedecea lapagei*	This patent	*tuf*
20	*Chlamydia pneumoniae*	This patent	*tuf*
21	*Chlamydia psittaci*	This patent	*tuf*
22	*Chlamydia trachomatis*	This patent	*tuf*
23	*Chryseobacterium meningosepticum*	This patent	*tuf*
24	*Citrobacter amalonaticus*	This patent	*tuf*
25	*Citrobacter braakii*	This patent	*tuf*
26	*Citrobacter koseri*	This patent	*tuf*
27	*Citrobacter farmeri*	This patent	*tuf*
28	*Citrobacter freundii*	This patent	*tuf*
29	*Citrobacter sedlakii*	This patent	*tuf*
30	*Citrobacter werkmanii*	This patent	*tuf*
31	*Citrobacter youngae*	This patent	*tuf*
32	*Clostridium perfringens*	This patent	*tuf*
33	*Comamonas acidovorans*	This patent	*tuf*
34	*Corynebacterium bovis*	This patent	*tuf*
35	*Corynebacterium cervicis*	This patent	*tuf*
36	*Corynebacterium flavescens*	This patent	*tuf*
37	*Corynebacterium kutscheri*	This patent	*tuf*
38	*Corynebacterium minutissimum*	This patent	*tuf*
39	*Corynebacterium mycetoides*	This patent	*tuf*
40	*Corynebacterium pseudogenitalium*	This patent	*tuf*
41	*Corynebacterium renale*	This patent	*tuf*
42	*Corynebacterium ulcerans*	This patent	*tuf*
43	*Corynebacterium urealyticum*	This patent	*tuf*
44	*Corynebacterium xerosis*	This patent	*tuf*
45	*Coxiella burnetii*	This patent	*tuf*
46	*Edwardsiella hoshinae*	This patent	*tuf*
47	*Edwardsiella tarda*	This patent	*tuf*
48	*Eikenella corrodens*	This patent	*tuf*
49	*Enterobacter aerogenes*	This patent	*tuf*
50	*Enterobacter agglomerans*	This patent	*tuf*
51	*Enterobacter amnigenus*	This patent	*tuf*
52	*Enterobacter asburiae*	This patent	*tuf*
53	*Enterobacter cancerogenus*	This patent	*tuf*
54	*Enterobacter cloacae*	This patent	*tuf*
55	*Enterobacter gergoviae*	This patent	*tuf*
56	*Enterobacter hormaechei*	This patent	*tuf*
57	*Enterobacter sakazakii*	This patent	*tuf*
58	*Enterococcus casseliflavus*	This patent	*tuf*
59	*Enterococcus cecorum*	This patent	*tuf*
60	*Enterococcus dispar*	This patent	*tuf*
61	*Enterococcus durans*	This patent	*tuf*

Table 7. Origin of the nucleic acids and/or sequences in the sequence listing (c ntinued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
62	*Enterococcus faecalis*	This patent	*tuf*
63	*Enterococcus faecalis*	This patent	*tuf*
64	*Enterococcus faecium*	This patent	*tuf*
65	*Enterococcus flavescens*	This patent	*tuf*
66	*Enterococcus gallinarum*	This patent	*tuf*
67	*Enterococcus hirae*	This patent	*tuf*
68	*Enterococcus mundtii*	This patent	*tuf*
69	*Enterococcus pseudoavium*	This patent	*tuf*
70	*Enterococcus raffinosus*	This patent	*tuf*
71	*Enterococcus saccharolyticus*	This patent	*tuf*
72	*Enterococcus solitarius*	This patent	*tuf*
73	*Enterococcus casseliflavus*	This patent	*tuf* (C)
74	*Staphylococcus saprophyticus*	This patent	unknown
75	*Enterococcus flavescens*	This patent	*tuf* (C)
76	*Enterococcus gallinarum*	This patent	*tuf* (C)
77	*Ehrlichia canis*	This patent	*tuf*
78	*Escherichia coli*	This patent	*tuf*
79	*Escherichia fergusonii*	This patent	*tuf*
80	*Escherichia hermannii*	This patent	*tuf*
81	*Escherichia vulneris*	This patent	*tuf*
82	*Eubacterium lentum*	This patent	*tuf*
83	*Eubacterium nodatum*	This patent	*tuf*
84	*Ewingella americana*	This patent	*tuf*
85	*Francisella tularensis*	This patent	*tuf*
86	*Fusobacterium nucleatum* subsp. *polymorphum*	This patent	*tuf*
87	*Gemella haemolysans*	This patent	*tuf*
88	*Gemella morbillorum*	This patent	*tuf*
89	*Haemophilus actinomycetemcomitans*	This patent	*tuf*
90	*Haemophilus aphrophilus*	This patent	*tuf*
91	*Haemophilus ducreyi*	This patent	*tuf*
92	*Haemophilus haemolyticus*	This patent	*tuf*
93	*Haemophilus parahaemolyticus*	This patent	*tuf*
94	*Haemophilus parainfluenzae*	This patent	*tuf*
95	*Haemophilus paraphrophilus*	This patent	*tuf*
96	*Haemophilus segnis*	This patent	*tuf*
97	*Hafnia alvei*	This patent	*tuf*
98	*Kingella kingae*	This patent	*tuf*
99	*Klebsiella ornithinolytica*	This patent	*tuf*
100	*Klebsiella oxytoca*	This patent	*tuf*
101	*Klebsiella planticola*	This patent	*tuf*
102	*Klebsiella pneumoniae* subsp. *ozaenae*	This patent	*tuf*
103	*Klebsiella pneumoniae pneumoniae*	This patent	*tuf*
104	*Klebsiella pneumoniae* subsp. *rhinoscleromatis*	This patent	*tuf*
105	*Kluyvera ascorbata*	This patent	*tuf*
106	*Kluyvera cryocrescens*	This patent	*tuf*
107	*Kluyvera georgiana*	This patent	*tuf*
108	*Lactobacillus casei subsp. casei*	This patent	*tuf*
109	*Lactococcus lactis* subsp. *lactis*	This patent	*tuf*
110	*Leclercia adecarboxylata*	This patent	*tuf*
111	*Legionella micdadei*	This patent	*tuf*
112	*Legionella pneumophila* subsp. *pneumophila*	This patent	*tuf*
113	*Leminorella grimontii*	This patent	*tuf*
114	*Leminorella richardii*	This patent	*tuf*
115	*Leptospira interrogans*	This patent	*tuf*
116	*Megamonas hypermegale*	This patent	*tuf*
117	*Mitsuokella multacidus*	This patent	*tuf*
118	*Mobiluncus curtisii* subsp. *holmesii*	This patent	*tuf*
119	*Moellerella wisconsensis*	This patent	*tuf*
120	*Moraxella catarrhalis*	This patent	*tuf*
121	*Morganella morganii* subsp. *morganii*	This patent	*tuf*
122	*Mycobacterium tuberculosis*	This patent	*tuf*

Table 7. Origin f the nucleic acids and/or sequences in the sequence listing (continued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
123	*Neisseria cinerea*	This patent	*tuf*
124	*Neisseria elongata* subsp. *elongata*	This patent	*tuf*
125	*Neisseria flavescens*	This patent	*tuf*
126	*Neisseria gonorrhoeae*	This patent	*tuf*
127	*Neisseria lactamica*	This patent	*tuf*
128	*Neisseria meningitidis*	This patent	*tuf*
129	*Neisseria mucosa*	This patent	*tuf*
130	*Neisseria sicca*	This patent	*tuf*
131	*Neisseria subflava*	This patent	*tuf*
132	*Neisseria weaveri*	This patent	*tuf*
133	*Ochrobactrum anthropi*	This patent	*tuf*
134	*Pantoea agglomerans*	This patent	*tuf*
135	*Pantoea dispersa*	This patent	*tuf*
136	*Pasteurella multocida*	This patent	*tuf*
137	*Peptostreptococcus anaerobius*	This patent	*tuf*
138	*Peptostreptococcus asaccharolyticus*	This patent	*tuf*
139	*Peptostreptococcus prevotii*	This patent	*tuf*
140	*Porphyromonas asaccharolytica*	This patent	*tuf*
141	*Porphyromonas gingivalis*	This patent	*tuf*
142	*Pragia fontium*	This patent	*tuf*
143	*Prevotella melaninogenica*	This patent	*tuf*
144	*Prevotella oralis*	This patent	*tuf*
145	*Propionibacterium acnes*	This patent	*tuf*
146	*Proteus mirabilis*	This patent	*tuf*
147	*Proteus penneri*	This patent	*tuf*
148	*Proteus vulgaris*	This patent	*tuf*
149	*Providencia alcalifaciens*	This patent	*tuf*
150	*Providencia rettgeri*	This patent	*tuf*
151	*Providencia rustigianii*	This patent	*tuf*
152	*Providencia stuartii*	This patent	*tuf*
153	*Pseudomonas aeruginosa*	This patent	*tuf*
154	*Pseudomonas fluorescens*	This patent	*tuf*
155	*Pseudomonas stutzeri*	This patent	*tuf*
156	*Psychrobacter phenylpyruvicum*	This patent	*tuf*
157	*Rahnella aquatilis*	This patent	*tuf*
158	*Salmonella choleraesuis* subsp.*arizonae*	This patent	*tuf*
159	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Choleraesuis	This patent	*tuf*
160	*Salmonella choleraesuis* subsp. *diarizonae*	This patent	*tuf*
161	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Heidelberg	This patent	*tuf*
162	*Salmonella choleraesuis* subsp. *houtenae*	This patent	*tuf*
163	*Salmonella choleraesuis* subsp. *indica*	This patent	*tuf*
164	*Salmonella choleraesuis* subsp. *salamae*	This patent	*tuf*
165	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Typhi	This patent	*tuf*
166	*Serratia fonticola*	This patent	*tuf*
167	*Serratia liquefaciens*	This patent	*tuf*
168	*Serratia marcescens*	This patent	*tuf*
169	*Serratia odorifera*	This patent	*tuf*
170	*Serratia plymuthica*	This patent	*tuf*
171	*Serratia rubidaea*	This patent	*tuf*
172	*Shigella boydii*	This patent	*tuf*
173	*Shigella dysenteriae*	This patent	*tuf*
174	*Shigella flexneri*	This patent	*tuf*
175	*Shigella sonnei*	This patent	*tuf*
176	*Staphylococcus aureus*	This patent	*tuf*
177	*Staphylococcus aureus*	This patent	*tuf*
178	*Staphylococcus aureus*	This patent	*tuf*
179	*Staphylococcus aureus*	This patent	*tuf*
180	*Staphylococcus aureus* subsp. *aureus*	This patent	*tuf*
181	*Staphylococcus auricularis*	This patent	*tuf*
182	*Staphylococcus capitis* subsp. *capitis*	This patent	*tuf*

Table 7. **Origin of the nucleic acids and/or sequences in the sequence listing (continued).**

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
183	*Macrococcus caseolyticus*	This patent	*tuf*
184	*Staphylococcus cohnii* subsp. *cohnii*	This patent	*tuf*
185	*Staphylococcus epidermidis*	This patent	*tuf*
186	*Staphylococcus haemolyticus*	This patent	*tuf*
187	*Staphylococcus warneri*	This patent	*tuf*
188	*Staphylococcus haemolyticus*	This patent	*tuf*
189	*Staphylococcus haemolyticus*	This patent	*tuf*
190	*Staphylococcus haemolyticus*	This patent	*tuf*
191	*Staphylococcus hominis* subsp. *hominis*	This patent	*tuf*
192	*Staphylococcus warneri*	This patent	*tuf*
193	*Staphylococcus hominis*	This patent	*tuf*
194	*Staphylococcus hominis*	This patent	*tuf*
195	*Staphylococcus hominis*	This patent	*tuf*
196	*Staphylococcus hominis*	This patent	*tuf*
197	*Staphylococcus lugdunensis*	This patent	*tuf*
198	*Staphylococcus saprophyticus*	This patent	*tuf*
199	*Staphylococcus saprophyticus*	This patent	*tuf*
200	*Staphylococcus saprophyticus*	This patent	*tuf*
201	*Staphylococcus sciuri* subsp. *sciuri*	This patent	*tuf*
202	*Staphylococcus warneri*	This patent	*tuf*
203	*Staphylococcus warneri*	This patent	*tuf*
204	*Bifidobacterium longum*	This patent	*tuf*
205	*Stenotrophomonas maltophilia*	This patent	*tuf*
206	*Streptococcus acidominimus*	This patent	*tuf*
207	*Streptococcus agalactiae*	This patent	*tuf*
208	*Streptococcus agalactiae*	This patent	*tuf*
209	*Streptococcus agalactiae*	This patent	*tuf*
210	*Streptococcus agalactiae*	This patent	*tuf*
211	*Streptococcus anginosus*	This patent	*tuf*
212	*Streptococcus bovis*	This patent	*tuf*
213	*Streptococcus anginosus*	This patent	*tuf*
214	*Streptococcus cricetus*	This patent	*tuf*
215	*Streptococcus cristatus*	This patent	*tuf*
216	*Streptococcus downei*	This patent	*tuf*
217	*Streptococcus dysgalactiae*	This patent	*tuf*
218	*Streptococcus equi* subsp. *equi*	This patent	*tuf*
219	*Streptococcus ferus*	This patent	*tuf*
220	*Streptococcus gordonii*	This patent	*tuf*
221	*Streptococcus anginosus*	This patent	*tuf*
222	*Streptococcus macacae*	This patent	*tuf*
223	*Streptococcus gordonii*	This patent	*tuf*
224	*Streptococcus mutans*	This patent	*tuf*
225	*Streptococcus parasanguinis*	This patent	*tuf*
226	*Streptococcus ratti*	This patent	*tuf*
227	*Streptococcus sanguinis*	This patent	*tuf*
228	*Streptococcus sobrinus*	This patent	*tuf*
229	*Streptococcus suis*	This patent	*tuf*
230	*Streptococcus uberis*	This patent	*tuf*
231	*Streptococcus vestibularis*	This patent	*tuf*
232	*Tatumella ptyseos*	This patent	*tuf*
233	*Trabulsiella guamensis*	This patent	*tuf*
234	*Veillonella parvula*	This patent	*tuf*
235	*Yersinia enterocolitica*	This patent	*tuf*
236	*Yersinia frederiksenii*	This patent	*tuf*
237	*Yersinia intermedia*	This patent	*tuf*
238	*Yersinia pestis*	This patent	*tuf*
239	*Yersinia pseudotuberculosis*	This patent	*tuf*
240	*Yersinia rohdei*	This patent	*tuf*
241	*Yokenella regensburgei*	This patent	*tuf*
242	*Achromobacter xylosoxidans* subsp. *denitrificans*	This patent	*atpD*
243	*Acinetobacter baumannii*	This patent	*atpD*
244	*Acinetobacter lwoffii*	This patent	*atpD*

Table 7. Origin of the nucleic acids and/or sequences in the sequence listing (continued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
245	*Staphylococcus saprophyticus*	This patent	*atpD*
246	*Alcaligenes faecalis* subsp. *faecalis*	This patent	*atpD*
247	*Bacillus anthracis*	This patent	*atpD*
248	*Bacillus cereus*	This patent	*atpD*
249	*Bacteroides distasonis*	This patent	*atpD*
250	*Bacteroides ovatus*	This patent	*atpD*
251	*Leclercia adecarboxylata*	This patent	*atpD*
252	*Stenotrophomonas maltophilia*	This patent	*atpD*
253	*Bartonella henselae*	This patent	*atpD*
254	*Bifidobacterium adolescentis*	This patent	*atpD*
255	*Brucella abortus*	This patent	*atpD*
256	*Cedecea davisae*	This patent	*atpD*
257	*Cedecea lapagei*	This patent	*atpD*
258	*Cedecea neteri*	This patent	*atpD*
259	*Chryseobacterium meningosepticum*	This patent	*atpD*
260	*Citrobacter amalonaticus*	This patent	*atpD*
261	*Citrobacter braakii*	This patent	*atpD*
262	*Citrobacter koseri*	This patent	*atpD*
263	*Citrobacter farmeri*	This patent	*atpD*
264	*Citrobacter freundii*	This patent	*atpD*
265	*Citrobacter koseri*	This patent	*atpD*
266	*Citrobacter sedlakii*	This patent	*atpD*
267	*Citrobacter werkmanii*	This patent	*atpD*
268	*Citrobacter youngae*	This patent	*atpD*
269	*Clostridium innocuum*	This patent	*atpD*
270	*Clostridium perfringens*	This patent	*atpD*
272	*Corynebacterium diphtheriae*	This patent	*atpD*
273	*Corynebacterium pseudodiphtheriticum*	This patent	*atpD*
274	*Corynebacterium ulcerans*	This patent	*atpD*
275	*Corynebacterium urealyticum*	This patent	*atpD*
276	*Coxiella burnetii*	This patent	*atpD*
277	*Edwardsiella hoshinae*	This patent	*atpD*
278	*Edwardsiella tarda*	This patent	*atpD*
279	*Eikenella corrodens*	This patent	*atpD*
280	*Enterobacter agglomerans*	This patent	*atpD*
281	*Enterobacter amnigenus*	This patent	*atpD*
282	*Enterobacter asburiae*	This patent	*atpD*
283	*Enterobacter cancerogenus*	This patent	*atpD*
284	*Enterobacter cloacae*	This patent	*atpD*
285	*Enterobacter gergoviae*	This patent	*atpD*
286	*Enterobacter hormaechei*	This patent	*atpD*
287	*Enterobacter sakazakii*	This patent	*atpD*
288	*Enterococcus avium*	This patent	*atpD*
289	*Enterococcus casseliflavus*	This patent	*atpD*
290	*Enterococcus durans*	This patent	*atpD*
291	*Enterococcus faecalis*	This patent	*atpD*
292	*Enterococcus faecium*	This patent	*atpD*
293	*Enterococcus gallinarum*	This patent	*atpD*
294	*Enterococcus saccharolyticus*	This patent	*atpD*
295	*Escherichia fergusonii*	This patent	*atpD*
296	*Escherichia hermannii*	This patent	*atpD*
297	*Escherichia vulneris*	This patent	*atpD*
298	*Eubacterium lentum*	This patent	*atpD*
299	*Ewingella americana*	This patent	*atpD*
300	*Francisella tularensis*	This patent	*atpD*
301	*Fusobacterium gonidiaformans*	This patent	*atpD*
302	*Fusobacterium necrophorum* subsp. *necrophorum*	This patent	*atpD*
303	*Fusobacterium nucleatum* subsp. *polymorphum*	This patent	*atpD*
304	*Gardnerella vaginalis*	This patent	*atpD*
305	*Gemella haemolysans*	This patent	*atpD*
306	*Gemella morbillorum*	This patent	*atpD*

Table 7. Origin of the nucleic acids and/ r sequences in the sequence listing (c ntinued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
307	*Haemophilus ducreyi*	This patent	*atpD*
308	*Haemophilus haemolyticus*	This patent	*atpD*
309	*Haemophilus parahaemolyticus*	This patent	*atpD*
310	*Haemophilus parainfluenzae*	This patent	*atpD*
311	*Hafnia alvei*	This patent	*atpD*
312	*Kingella kingae*	This patent	*atpD*
313	*Klebsiella pneumoniae* subsp. *ozaenae*	This patent	*atpD*
314	*Klebsiella ornithinolytica*	This patent	*atpD*
315	*Klebsiella oxytoca*	This patent	*atpD*
316	*Klebsiella planticola*	This patent	*atpD*
317	*Klebsiella pneumoniae* subsp. *pneumoniae*	This patent	*atpD*
318	*Kluyvera ascorbata*	This patent	*atpD*
319	*Kluyvera cryocrescens*	This patent	*atpD*
320	*Kluyvera georgiana*	This patent	*atpD*
321	*Lactobacillus acidophilus*	This patent	*atpD*
322	*Legionella pneumophila* subsp. *pneumophila*	This patent	*atpD*
323	*Leminorella grimontii*	This patent	*atpD*
324	*Listeria monocytogenes*	This patent	*atpD*
325	*Micrococcus lylae*	This patent	*atpD*
326	*Moellerella wisconsensis*	This patent	*atpD*
327	*Moraxella catarrhalis*	This patent	*atpD*
328	*Moraxella osloensis*	This patent	*atpD*
329	*Morganella morganii* subsp. *morganii*	This patent	*atpD*
330	*Pantoea agglomerans*	This patent	*atpD*
331	*Pantoea dispersa*	This patent	*atpD*
332	*Pasteurella multocida*	This patent	*atpD*
333	*Pragia fontium*	This patent	*atpD*
334	*Proteus mirabilis*	This patent	*atpD*
335	*Proteus vulgaris*	This patent	*atpD*
336	*Providencia alcalifaciens*	This patent	*atpD*
337	*Providencia rettgeri*	This patent	*atpD*
338	*Providencia rustigianii*	This patent	*atpD*
339	*Providencia stuartii*	This patent	*atpD*
340	*Psychrobacter phenylpyruvicum*	This patent	*atpD*
341	*Rahnella aquatilis*	This patent	*atpD*
342	*Salmonella choleraesuis* subsp. *arizonae*	This patent	*atpD*
343	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Choleraesuis	This patent	*atpD*
344	*Salmonella choleraesuis* subsp. *diarizonae*	This patent	*atpD*
345	*Salmonella choleraesuis* subsp. *houtenae*	This patent	*atpD*
346	*Salmonella choleraesuis* subsp. *indica*	This patent	*atpD*
347	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Paratyphi A	This patent	*atpD*
348	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Paratyphi B	This patent	*atpD*
349	*Salmonella choleraesuis* subsp. *salamae*	This patent	*atpD*
350	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Typhi	This patent	*atpD*
351	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Typhimurium	This patent	*atpD*
352	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Virchow	This patent	*atpD*
353	*Serratia ficaria*	This patent	*atpD*
354	*Serratia fonticola*	This patent	*atpD*
355	*Serratia grimesii*	This patent	*atpD*
356	*Serratia liquefaciens*	This patent	*atpD*
357	*Serratia marcescens*	This patent	*atpD*
358	*Serratia odorifera*	This patent	*atpD*
359	*Serratia plymuthica*	This patent	*atpD*
360	*Serratia rubidaea*	This patent	*atpD*
361	*Pseudomonas putida*	This patent	*atpD*
362	*Shigella boydii*	This patent	*atpD*
363	*Shigella dysenteriae*	This patent	*atpD*

Table 7. Origin of the nucleic acids and/or sequences in the sequence listing (continued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
364	*Shigella flexneri*	This patent	*atpD*
365	*Shigella sonnei*	This patent	*atpD*
366	*Staphylococcus aureus*	This patent	*atpD*
367	*Staphylococcus auricularis*	This patent	*atpD*
368	*Staphylococcus capitis* subsp. *capitis*	This patent	*atpD*
369	*Staphylococcus cohnii* subsp. *cohnii*	This patent	*atpD*
370	*Staphylococcus epidermidis*	This patent	*atpD*
371	*Staphylococcus haemolyticus*	This patent	*atpD*
372	*Staphylococcus hominis* subsp. *hominis*	This patent	*atpD*
373	*Staphylococcus hominis*	This patent	*atpD*
374	*Staphylococcus lugdunensis*	This patent	*atpD*
375	*Staphylococcus saprophyticus*	This patent	*atpD*
376	*Staphylococcus simulans*	This patent	*atpD*
377	*Staphylococcus warneri*	This patent	*atpD*
378	*Streptococcus acidominimus*	This patent	*atpD*
379	*Streptococcus agalactiae*	This patent	*atpD*
380	*Streptococcus agalactiae*	This patent	*atpD*
381	*Streptococcus agalactiae*	This patent	*atpD*
382	*Streptococcus agalactiae*	This patent	*atpD*
383	*Streptococcus agalactiae*	This patent	*atpD*
384	*Streptococcus dysgalactiae*	This patent	*atpD*
385	*Streptococcus equi* subsp. *equi*	This patent	*atpD*
386	*Streptococcus anginosus*	This patent	*atpD*
387	*Streptococcus salivarius*	This patent	*atpD*
388	*Streptococcus suis*	This patent	*atpD*
389	*Streptococcus uberis*	This patent	*atpD*
390	*Tatumella ptyseos*	This patent	*atpD*
391	*Trabulsiella guamensis*	This patent	*atpD*
392	*Yersinia bercovieri*	This patent	*atpD*
393	*Yersinia enterocolitica*	This patent	*atpD*
394	*Yersinia frederiksenii*	This patent	*atpD*
395	*Yersinia intermedia*	This patent	*atpD*
396	*Yersinia pseudotuberculosis*	This patent	*atpD*
397	*Yersinia rohdei*	This patent	*atpD*
398	*Yokenella regensburgei*	This patent	*atpD*
399	*Yarrowia lipolytica*	This patent	*tuf* (EF-1)
400	*Absidia corymbifera*	This patent	*tuf* (EF-1)
401	*Alternaria alternata*	This patent	*tuf* (EF-1)
402	*Aspergillus flavus*	This patent	*tuf* (EF-1)
403	*Aspergillus fumigatus*	This patent	*tuf* (EF-1)
404	*Aspergillus fumigatus*	This patent	*tuf* (EF-1)
405	*Aspergillus niger*	This patent	*tuf* (EF-1)
406	*Blastoschizomyces capitatus*	This patent	*tuf* (EF-1)
407	*Candida albicans*	This patent	*tuf* (EF-1)
408	*Candida albicans*	This patent	*tuf* (EF-1)
409	*Candida albicans*	This patent	*tuf* (EF-1)
410	*Candida albicans*	This patent	*tuf* (EF-1)
411	*Candida albicans*	This patent	*tuf* (EF-1)
412	*Candida dubliniensis*	This patent	*tuf* (EF-1)
413	*Candida catenulata*	This patent	*tuf* (EF-1)
414	*Candida dubliniensis*	This patent	*tuf* (EF-1)
415	*Candida dubliniensis*	This patent	*tuf* (EF-1)
416	*Candida famata*	This patent	*tuf* (EF-1)
417	*Candida glabrata*	WO98/20157	*tuf* (EF-1)
418	*Candida guilliermondii*	This patent	*tuf* (EF-1)
419	*Candida haemulonii*	This patent	*tuf* (EF-1)
420	*Candida inconspicua*	This patent	*tuf* (EF-1)
421	*Candida kefyr*	This patent	*tuf* (EF-1)
422	*Candida krusei*	WO98/20157	*tuf* (EF-1)
423	*Candida lambica*	This patent	*tuf* (EF-1)
424	*Candida lusitaniae*	This patent	*tuf* (EF-1)
425	*Candida norvegensis*	This patent	*tuf* (EF-1)

Table 7. Origin of the nucleic acids and/or sequences in the sequence listing (c ntinued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
426	*Candida parapsilosis*	WO98/20157	*tuf* (EF-1)
427	*Candida rugosa*	This patent	*tuf* (EF-1)
428	*Candida sphaerica*	This patent	*tuf* (EF-1)
429	*Candida tropicalis*	WO98/20157	*tuf* (EF-1)
430	*Candida utilis*	This patent	*tuf* (EF-1)
431	*Candida viswanathii*	This patent	*tuf* (EF-1)
432	*Candida zeylanoides*	This patent	*tuf* (EF-1)
433	*Coccidioides immitis*	This patent	*tuf* (EF-1)
434	*Cryptococcus albidus*	This patent	*tuf* (EF-1)
435	*Exophiala jeanselmei*	This patent	*tuf* (EF-1)
436	*Fusarium oxysporum*	This patent	*tuf* (EF-1)
437	*Geotrichum* sp.	This patent	*tuf* (EF-1)
438	*Histoplasma capsulatum*	This patent	*tuf* (EF-1)
439	*Issatchenkia orientalis* Kudrjanzev	This patent	*tuf* (EF-1)
440	*Malassezia furfur*	This patent	*tuf* (EF-1)
441	*Malassezia pachydermatis*	This patent	*tuf* (EF-1)
442	*Malbranchea filamentosa*	This patent	*tuf* (EF-1)
443	*Metschnikowia pulcherrima*	This patent	*tuf* (EF-1)
444	*Paecilomyces lilacinus*	This patent	*tuf* (EF-1)
445	*Paracoccidioides brasiliensis*	This patent	*tuf* (EF-1)
446	*Penicillium marneffei*	This patent	*tuf* (EF-1)
447	*Pichia anomala*	This patent	*tuf* (EF-1)
448	*Pichia anomala*	This patent	*tuf* (EF-1)
449	*Pseudallescheria boydii*	This patent	*tuf* (EF-1)
450	*Rhizopus oryzae*	This patent	*tuf* (EF-1)
451	*Rhodotorula minuta*	This patent	*tuf* (EF-1)
452	*Sporobolomyces salmonicolor*	This patent	*tuf* (EF-1)
453	*Sporothrix schenckii*	This patent	*tuf* (EF-1)
454	*Stephanoascus ciferrii*	This patent	*tuf* (EF-1)
455	*Trichophyton mentagrophytes*	This patent	*tuf* (EF-1)
456	*Trichosporon cutaneum*	This patent	*tuf* (EF-1)
457	*Wangiella dermatitidis*	This patent	*tuf* (EF-1)
458	*Aspergillus fumigatus*	This patent	*atpD*
459	*Blastoschizomyces capitatus*	This patent	*atpD*
460	*Candida albicans*	This patent	*atpD*
461	*Candida dubliniensis*	This patent	*atpD*
462	*Candida famata*	This patent	*atpD*
463	*Candida glabrata*	This patent	*atpD*
464	*Candida guilliermondii*	This patent	*atpD*
465	*Candida haemulonii*	This patent	*atpD*
466	*Candida inconspicua*	This patent	*atpD*
467	*Candida kefyr*	This patent	*atpD*
468	*Candida krusei*	This patent	*atpD*
469	*Candida lambica*	This patent	*atpD*
470	*Candida lusitaniae*	This patent	*atpD*
471	*Candida norvegensis*	This patent	*atpD*
472	*Candida parapsilosis*	This patent	*atpD*
473	*Candida rugosa*	This patent	*atpD*
474	*Candida sphaerica*	This patent	*atpD*
475	*Candida tropicalis*	This patent	*atpD*
476	*Candida utilis*	This patent	*atpD*
477	*Candida viswanathii*	This patent	*atpD*
478	*Candida zeylanoides*	This patent	*atpD*
479	*Coccidioides immitis*	This patent	*atpD*
480	*Cryptococcus albidus*	This patent	*atpD*
481	*Fusarium oxysporum*	This patent	*atpD*
482	*Geotrichum* sp.	This patent	*atpD*
483	*Histoplasma capsulatum*	This patent	*atpD*
484	*Malassezia furfur*	This patent	*atpD*
485	*Malassezia pachydermatis*	This patent	*atpD*
486	*Metschnikowia pulcherrima*	This patent	*atpD*
487	*Penicillium marneffei*	This patent	*atpD*

Table 7. Origin f the nucleic acids and/ r sequences in the sequence listing (c ntinued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
488	*Pichia anomala*	This patent	*atpD*
489	*Pichia anomala*	This patent	*atpD*
490	*Rhodotorula minuta*	This patent	*atpD*
491	*Rhodotorula mucilaginosa*	This patent	*atpD*
492	*Sporobolomyces salmonicolor*	This patent	*atpD*
493	*Sporothrix schenckii*	This patent	*atpD*
494	*Stephanoascus ciferrii*	This patent	*atpD*
495	*Trichophyton mentagrophytes*	This patent	*atpD*
496	*Wangiella dermatitidis*	This patent	*atpD*
497	*Yarrowia lipolytica*	This patent	*atpD*
498	*Aspergillus fumigatus*	This patent	*tuf* (M)
499	*Blastoschizomyces capitatus*	This patent	*tuf* (M)
500	*Candida rugosa*	This patent	*tuf* (M)
501	*Coccidioides immitis*	This patent	*tuf* (M)
502	*Fusarium oxysporum*	This patent	*tuf* (M)
503	*Histoplasma capsulatum*	This patent	*tuf* (M)
504	*Paracoccidioides brasiliensis*	This patent	*tuf* (M)
505	*Penicillium marneffei*	This patent	*tuf* (M)
506	*Pichia anomala*	This patent	*tuf* (M)
507	*Trichophyton mentagrophytes*	This patent	*tuf* (M)
508	*Yarrowia lipolytica*	This patent	*tuf* (M)
509	*Babesia bigemina*	This patent	*tuf* (EF-1)
510	*Babesia bovis*	This patent	*tuf* (EF-1)
511	*Crithidia fasciculata*	This patent	*tuf* (EF-1)
512	*Entamoeba histolytica*	This patent	*tuf* (EF-1)
513	*Giardia lamblia*	This patent	*tuf* (EF-1)
514	*Leishmania tropica*	This patent	*tuf* (EF-1)
515	*Leishmania aethiopica*	This patent	*tuf* (EF-1)
516	*Leishmania tropica*	This patent	*tuf* (EF-1)
517	*Leishmania donovani*	This patent	*tuf* (EF-1)
518	*Leishmania infantum*	This patent	*tuf* (EF-1)
519	*Leishmania enriettii*	This patent	*tuf* (EF-1)
520	*Leishmania gerbilli*	This patent	*tuf* (EF-1)
521	*Leishmania hertigi*	This patent	*tuf* (EF-1)
522	*Leishmania major*	This patent	*tuf* (EF-1)
523	*Leishmania amazonensis*	This patent	*tuf* (EF-1)
524	*Leishmania mexicana*	This patent	*tuf* (EF-1)
525	*Leishmania tarentolae*	This patent	*tuf* (EF-1)
526	*Leishmania tropica*	This patent	*tuf* (EF-1)
527	*Neospora caninum*	This patent	*tuf* (EF-1)
528	*Trichomonas vaginalis*	This patent	*tuf* (EF-1)
529	*Trypanosoma brucei* subsp. *brucei*	This patent	*tuf* (EF-1)
530	*Crithidia fasciculata*	This patent	*atpD*
531	*Leishmania tropica*	This patent	*atpD*
532	*Leishmania aethiopica*	This patent	*atpD*
533	*Leishmania donovani*	This patent	*atpD*
534	*Leishmania infantum*	This patent	*atpD*
535	*Leishmania gerbilli*	This patent	*atpD*
536	*Leishmania hertigi*	This patent	*atpD*
537	*Leishmania major*	This patent	*atpD*
538	*Leishmania amazonensis*	This patent	*atpD*
607	*Enterococcus faecalis*	WO98/20157	*tuf*
608	*Enterococcus faecium*	WO98/20157	*tuf*
609	*Enterococcus gallinarum*	WO98/20157	*tuf*
610	*Haemophilus influenzae*	WO98/20157	*tuf*
611	*Staphylococcus epidermidis*	WO98/20157	*tuf*
612	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Paratyphi A	This patent	*tuf*
613	*Serratia ficaria*	This patent	*tuf*
614	*Enterococcus malodoratus*	This patent	*tuf* (C)
615	*Enterococcus durans*	This patent	*tuf* (C)
616	*Enterococcus pseudoavium*	This patent	*tuf* (C)

Table 7. Origin f the nucleic acids and/ r sequences in the sequence listing (continued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
617	*Enterococcus dispar*	This patent	*tuf* (C)
618	*Enterococcus avium*	This patent	*tuf* (C)
619	*Saccharomyces cerevisiae*	Database	*tuf* (M)
621	*Enterococcus faecium*	This patent	*tuf* (C)
622	*Saccharomyces cerevisiae*	This patent	*tuf* (EF-1)
623	*Cryptococcus neoformans*	This patent	*tuf* (EF-1)
624	*Candida albicans*	WO98/20157	*tuf* (EF-1)
662	*Corynebacterium diphtheriae*	WO98/20157	*tuf*
663	*Candida catenulata*	This patent	*atpD*
665	*Saccharomyces cerevisiae*	Database	*tuf* (EF-1)
666	*Saccharomyces cerevisiae*	Database	*atpD*
667	*Trypanosoma cruzi*	This patent	*atpD*
668	*Corynebacterium glutamicum*	Database	*tuf*
669	*Escherichia coli*	Database	*atpD*
670	*Helicobacter pylori*	Database	*atpD*
671	*Clostridium acetobutylicum*	Database	*atpD*
672	*Cytophaga lytica*	Database	*atpD*
673	*Ehrlichia risticii*	This patent	*atpD*
674	*Vibrio cholerae*	This patent	*atpD*
675	*Vibrio cholerae*	This patent	*tuf*
676	*Leishmania enriettii*	This patent	*atpD*
677	*Babesia microti*	This patent	*tuf* (EF-1)
678	*Cryptococcus neoformans*	This patent	*atpD*
679	*Cryptococcus neoformans*	This patent	*atpD*
680	*Cunninghamella bertholletiae*	This patent	*atpD*
684	*Candida tropicalis*	Database	*atpD* (V)
685	*Enterococcus hirae*	Database	*atpD* (V)
686	*Chlamydia pneumoniae*	Database	*atpD* (V)
687	*Halobacterium salinarum*	Database	*atpD* (V)
688	*Homo sapiens*	Database	*atpD* (V)
689	*Plasmodium falciparum*	Database	*atpD* (V)
690	*Saccharomyces cerevisiae*	Database	*atpD* (V)
691	*Schizosaccharomyces pombe*	Database	*atpD* (V)
692	*Trypanosoma congolense*	Database	*atpD* (V)
693	*Thermus thermophilus*	Database	*atpD* (V)
698	*Escherichia coli*	WO98/20157	*tuf*
709	*Borrelia burgdorferi*	Database	*atpD* (V)
710	*Treponema pallidum*	Database	*atpD* (V)
711	*Chlamydia trachomatis*	Genome project	*atpD* (V)
712	*Enterococcus faecalis*	Genome project	*atpD* (V)
713	*Methanosarcina barkeri*	Database	*atpD* (V)
714	*Methanococcus jannaschii*	Database	*atpD* (V)
715	*Porphyromonas gingivalis*	Genome project	*atpD* (V)
716	*Streptococcus pneumoniae*	Genome project	*atpD* (V)
717	*Burkholderia mallei*	This patent	*tuf*
718	*Burkholderia pseudomallei*	This patent	*tuf*
719	*Clostridium beijerinckii*	This patent	*tuf*
720	*Clostridium innocuum*	This patent	*tuf*
721	*Clostridium novyi*	This patent	*tuf*
722	*Clostridium septicum*	This patent	*tuf*
723	*Clostridium tertium*	This patent	*tuf*
724	*Clostridium tetani*	This patent	*tuf*
725	*Enterococcus malodoratus*	This patent	*tuf*
726	*Enterococcus sulfureus*	This patent	*tuf*
727	*Lactococcus garvieae*	This patent	*tuf*
728	*Mycoplasma pirum*	This patent	*tuf*
729	*Mycoplasma salivarium*	This patent	*tuf*
730	*Neisseria polysaccharea*	This patent	*tuf*
731	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Enteritidis	This patent	*tuf*

Table 7. **Origin of the nucleic acids and/ r sequences in the sequence listing (continued).**

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
732	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Gallinarum	This patent	*tuf*
733	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Paratyphi B	This patent	*tuf*
734	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Virchow	This patent	*tuf*
735	*Serratia grimesii*	This patent	*tuf*
736	*Clostridium difficile*	This patent	*tuf*
737	*Burkholderia pseudomallei*	This patent	*atpD*
738	*Clostridium bifermentans*	This patent	*atpD*
739	*Clostridium beijerinckii*	This patent	*atpD*
740	*Clostridium difficile*	This patent	*atpD*
741	*Clostridium ramosum*	This patent	*atpD*
742	*Clostridium septicum*	This patent	*atpD*
743	*Clostridium tertium*	This patent	*atpD*
744	*Comamonas acidovorans*	This patent	*atpD*
745	*Klebsiella pneumoniae* subsp. *rhinoscleromatis*	This patent	*atpD*
746	*Neisseria canis*	This patent	*atpD*
747	*Neisseria cinerea*	This patent	*atpD*
748	*Neisseria cuniculi*	This patent	*atpD*
749	*Neisseria elongata* subsp. *elongata*	This patent	*atpD*
750	*Neisseria flavescens*	This patent	*atpD*
751	*Neisseria gonorrhoeae*	This patent	*atpD*
752	*Neisseria gonorrhoeae*	This patent	*atpD*
753	*Neisseria lactamica*	This patent	*atpD*
754	*Neisseria meningitidis*	This patent	*atpD*
755	*Neisseria mucosa*	This patent	*atpD*
756	*Neisseria subflava*	This patent	*atpD*
757	*Neisseria weaveri*	This patent	*atpD*
758	*Neisseria animalis*	This patent	*atpD*
759	*Proteus penneri*	This patent	*atpD*
760	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Enteritidis	This patent	*atpD*
761	*Yersinia pestis*	This patent	*atpD*
762	*Burkholderia mallei*	This patent	*atpD*
763	*Clostridium sordellii*	This patent	*atpD*
764	*Clostridium novyi*	This patent	*atpD*
765	*Clostridium botulinum*	This patent	*atpD*
766	*Clostridium histolyticum*	This patent	*atpD*
767	*Peptostreptococcus prevotii*	This patent	*atpD*
768	*Absidia corymbifera*	This patent	*atpD*
769	*Alternaria alternata*	This patent	*atpD*
770	*Aspergillus flavus*	This patent	*atpD*
771	*Mucor circinelloides*	This patent	*atpD*
772	*Piedraia hortai*	This patent	*atpD*
773	*Pseudallescheria boydii*	This patent	*atpD*
774	*Rhizopus oryzae*	This patent	*atpD*
775	*Scopulariopsis koningii*	This patent	*atpD*
776	*Trichophyton mentagrophytes*	This patent	*atpD*
777	*Trichophyton tonsurans*	This patent	*atpD*
778	*Trichosporon cutaneum*	This patent	*atpD*
779	*Cladophialophora carrionii*	This patent	*tuf* (EF-1)
780	*Cunninghamella bertholletiae*	This patent	*tuf* (EF-1)
781	*Curvularia lunata*	This patent	*tuf* (EF-1)
782	*Fonsecaea pedrosoi*	This patent	*tuf* (EF-1)
783	*Microsporum audouinii*	This patent	*tuf* (EF-1)
784	*Mucor circinelloides*	This patent	*tuf* (EF-1)
785	*Phialophora verrucosa*	This patent	*tuf* (EF-1)
786	*Saksenaea vasiformis*	This patent	*tuf* (EF-1)
787	*Syncephalastrum racemosum*	This patent	*tuf* (EF-1)
788	*Trichophyton tonsurans*	This patent	*tuf* (EF-1)
789	*Trichophyton mentagrophytes*	This patent	*tuf* (EF-1)

Table 7. Origin of the nucleic acids and/or sequences in the sequence listing (c ntinued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
790	*Bipolaris hawaiiensis*	This patent	*tuf* (EF-1)
791	*Aspergillus fumigatus*	This patent	*tuf* (M)
792	*Trichophyton mentagrophytes*	This patent	*tuf* (M)
827	*Clostridium novyi*	This patent	*atpD* (V)
828	*Clostridium difficile*	This patent	*atpD* (V)
829	*Clostridium septicum*	This patent	*atpD* (V)
830	*Clostridium botulinum*	This patent	*atpD* (V)
831	*Clostridium perfringens*	This patent	*atpD* (V)
832	*Clostridium tetani*	This patent	*atpD* (V)
833	*Streptococcus pyogenes*	Database	*atpD* (V)
834	*Babesia bovis*	This patent	*atpD* (V)
835	*Cryptosporidium parvum*	This patent	*atpD* (V)
836	*Leishmania infantum*	This patent	*atpD* (V)
837	*Leishmania major*	This patent	*atpD* (V)
838	*Leishmania tarentolae*	This patent	*atpD* (V)
839	*Trypanosoma brucei*	This patent	*atpD* (V)
840	*Trypanosoma cruzi*	This patent	*tuf* (EF-1)
841	*Trypanosoma cruzi*	This patent	*tuf* (EF-1)
842	*Trypanosoma cruzi*	This patent	*tuf* (EF-1)
843	*Babesia bovis*	This patent	*tuf* (M)
844	*Leishmania aethiopica*	This patent	*tuf* (M)
845	*Leishmania amazonensis*	This patent	*tuf* (M)
846	*Leishmania donovani*	This patent	*tuf* (M)
847	*Leishmania infantum*	This patent	*tuf* (M)
848	*Leishmania enriettii*	This patent	*tuf* (M)
849	*Leishmania gerbilli*	This patent	*tuf* (M)
850	*Leishmania major*	This patent	*tuf* (M)
851	*Leishmania mexicana*	This patent	*tuf* (M)
852	*Leishmania tarentolae*	This patent	*tuf* (M)
853	*Trypanosoma cruzi*	This patent	*tuf* (M)
854	*Trypanosoma cruzi*	This patent	*tuf* (M)
855	*Trypanosoma cruzi*	This patent	*tuf* (M)
856	*Babesia bigemina*	This patent	*atpD*
857	*Babesia bovis*	This patent	*atpD*
858	*Babesia microti*	This patent	*atpD*
859	*Leishmania guyanensis*	This patent	*atpD*
860	*Leishmania mexicana*	This patent	*atpD*
861	*Leishmania tropica*	This patent	*atpD*
862	*Leishmania tropica*	This patent	*atpD*
863	*Bordetella pertussis*	Database	*tuf*
864	*Trypanosoma brucei brucei*	Database	*tuf* (EF-1)
865	*Cryptosporidium parvum*	This patent	*tuf* (EF-1)
866	*Staphylococcus saprophyticus*	This patent	*atpD*
867	*Zoogloea ramigera*	This patent	*atpD*
868	*Staphylococcus saprophyticus*	This patent	*tuf*
869	*Enterococcus casseliflavus*	This patent	*tuf*
870	*Enterococcus casseliflavus*	This patent	*tuf*
871	*Enterococcus flavescens*	This patent	*tuf*
872	*Enterococcus gallinarum*	This patent	*tuf*
873	*Enterococcus gallinarum*	This patent	*tuf*
874	*Staphylococcus haemolyticus*	This patent	*tuf*
875	*Staphylococcus epidermidis*	This patent	*tuf*
876	*Staphylococcus epidermidis*	This patent	*tuf*
877	*Staphylococcus epidermidis*	This patent	*tuf*
878	*Staphylococcus epidermidis*	This patent	*tuf*
879	*Enterococcus gallinarum*	This patent	*tuf*
880	*Pseudomonas aeruginosa*	This patent	*tuf*
881	*Enterococcus casseliflavus*	This patent	*tuf*
882	*Enterococcus casseliflavus*	This patent	*tuf*
883	*Enterococcus faecalis*	This patent	*tuf*
884	*Enterococcus faecalis*	This patent	*tuf*
885	*Enterococcus faecium*	This patent	*tuf*

Table 7. Origin f the nucleic acids and/or sequences in the sequence listing (c ntinued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
886	*Enterococcus faecium*	This patent	*tuf*
887	*Zoogloea ramigera*	This patent	*tuf*
888	*Enterococcus faecalis*	This patent	*tuf*
889	*Aspergillus fumigatus*	This patent	*atpD*
890	*Penicillium marneffei*	This patent	*atpD*
891	*Paecilomyces lilacinus*	This patent	*atpD*
892	*Penicillium marneffei*	This patent	*atpD*
893	*Sporothrix schenckii*	This patent	*atpD*
894	*Malbranchea filamentosa*	This patent	*atpD*
895	*Paecilomyces lilacinus*	This patent	*atpD*
896	*Aspergillus niger*	This patent	*atpD*
897	*Aspergillus fumigatus*	This patent	*tuf* (EF-1)
898	*Penicillium marneffei*	This patent	*tuf* (EF-1)
899	*Piedraia hortai*	This patent	*tuf* (EF-1)
900	*Paecilomyces lilacinus*	This patent	*tuf* (EF-1)
901	*Paracoccidioides brasiliensis*	This patent	*tuf* (EF-1)
902	*Sporothrix schenckii*	This patent	*tuf* (EF-1)
903	*Penicillium marneffei*	This patent	*tuf* (EF-1)
904	*Curvularia lunata*	This patent	*tuf* (M)
905	*Aspergillus niger*	This patent	*tuf* (M)
906	*Bipolaris hawaiiensis*	This patent	*tuf* (M)
907	*Aspergillus flavus*	This patent	*tuf* (M)
908	*Alternaria alternata*	This patent	*tuf* (M)
909	*Penicillium marneffei*	This patent	*tuf* (M)
910	*Penicillium marneffei*	This patent	*tuf* (M)
918	*Escherichia coli*	Database	*recA*
929	*Bacteroides fragilis*	This patent	*atpD* (V)
930	*Bacteroides distasonis*	This patent	*atpD* (V)
931	*Porphyromonas asaccharolytica*	This patent	*atpD* (V)
932	*Listeria monocytogenes*	This patent	*tuf*
939	*Saccharomyces cerevisiae*	Database	*recA* (Rad51)
940	*Saccharomyces cerevisiae*	Database	*recA* (Dmc1)
941	*Cryptococcus humicolus*	This patent	*atpD*
942	*Escherichia coli*	This patent	*atpD*
943	*Escherichia coli*	This patent	*atpD*
944	*Escherichia coli*	This patent	*atpD*
945	*Escherichia coli*	This patent	*atpD*
946	*Neisseria polysaccharea*	This patent	*atpD*
947	*Neisseria sicca*	This patent	*atpD*
948	*Streptococcus mitis*	This patent	*atpD*
949	*Streptococcus mitis*	This patent	*atpD*
950	*Streptococcus mitis*	This patent	*atpD*
951	*Streptococcus oralis*	This patent	*atpD*
952	*Streptococcus pneumoniae*	This patent	*atpD*
953	*Streptococcus pneumoniae*	This patent	*atpD*
954	*Streptococcus pneumoniae*	This patent	*atpD*
955	*Streptococcus pneumoniae*	This patent	*atpD*
956	*Babesia microti*	This patent	*atpD* (V)
957	*Entamoeba histolytica*	This patent	*atpD* (V)
958	*Fusobacterium nucleatum* subsp. *polymorphum*	This patent	*atpD* (V)
959	*Leishmania aethiopica*	This patent	*atpD* (V)
960	*Leishmania tropica*	This patent	*atpD* (V)
961	*Leishmania guyanensis*	This patent	*atpD* (V)
962	*Leishmania donovani*	This patent	*atpD* (V)
963	*Leishmania hertigi*	This patent	*atpD* (V)
964	*Leishmania mexicana*	This patent	*atpD* (V)
965	*Leishmania tropica*	This patent	*atpD* (V)
966	*Peptostreptococcus anaerobius*	This patent	*atpD* (V)
967	*Bordetella pertussis*	This patent	*tuf*
968	*Bordetella pertussis*	This patent	*tuf*
969	*Enterococcus columbae*	This patent	*tuf*

Table 7. Origin f the nucleic acids and/or sequences in the sequence listing (continued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
970	*Enterococcus flavescens*	This patent	*tuf*
971	*Streptococcus pneumoniae*	This patent	*tuf*
972	*Escherichia coli*	This patent	*tuf*
973	*Escherichia coli*	This patent	*tuf*
974	*Escherichia coli*	This patent	*tuf*
975	*Escherichia coli*	This patent	*tuf*
976	*Mycobacterium avium*	This patent	*tuf*
977	*Streptococcus pneumoniae*	This patent	*tuf*
978	*Mycobacterium gordonae*	This patent	*tuf*
979	*Streptococcus pneumoniae*	This patent	*tuf*
980	*Mycobacterium tuberculosis*	This patent	*tuf*
981	*Staphylococcus warneri*	This patent	*tuf*
982	*Streptococcus mitis*	This patent	*tuf*
983	*Streptococcus mitis*	This patent	*tuf*
984	*Streptococcus mitis*	This patent	*tuf*
985	*Streptococcus oralis*	This patent	*tuf*
986	*Streptococcus pneumoniae*	This patent	*tuf*
987	*Enterococcus hirae*	This patent	*tuf* (C)
988	*Enterococcus mundtii*	This patent	*tuf* (C)
989	*Enterococcus raffinosus*	This patent	*tuf* (C)
990	*Bacillus anthracis*	This patent	*recA*
991	*Prevotella melaninogenica*	This patent	*recA*
992	*Enterococcus casseliflavus*	This patent	*tuf*
993	*Streptococcus pyogenes*	Database	*speA*
1002	*Streptococcus pyogenes*	WO98/20157	*tuf*
1003	*Bacillus cereus*	This patent	*recA*
1004	*Streptococcus pneumoniae*	This patent	*pbp1a*
1005	*Streptococcus pneumoniae*	This patent	*pbp1a*
1006	*Streptococcus pneumoniae*	This patent	*pbp1a*
1007	*Streptococcus pneumoniae*	This patent	*pbp1a*
1008	*Streptococcus pneumoniae*	This patent	*pbp1a*
1009	*Streptococcus pneumoniae*	This patent	*pbp1a*
1010	*Streptococcus pneumoniae*	This patent	*pbp1a*
1011	*Streptococcus pneumoniae*	This patent	*pbp1a*
1012	*Streptococcus pneumoniae*	This patent	*pbp1a*
1013	*Streptococcus pneumoniae*	This patent	*pbp1a*
1014	*Streptococcus pneumoniae*	This patent	*pbp1a*
1015	*Streptococcus pneumoniae*	This patent	*pbp1a*
1016	*Streptococcus pneumoniae*	This patent	*pbp1a*
1017	*Streptococcus pneumoniae*	This patent	*pbp1a*
1018	*Streptococcus pneumoniae*	This patent	*pbp1a*
1019	*Streptococcus pneumoniae*	This patent	*pbp2b*
1020	*Streptococcus pneumoniae*	This patent	*pbp2b*
1021	*Streptococcus pneumoniae*	This patent	*pbp2b*
1022	*Streptococcus pneumoniae*	This patent	*pbp2b*
1023	*Streptococcus pneumoniae*	This patent	*pbp2b*
1024	*Streptococcus pneumoniae*	This patent	*pbp2b*
1025	*Streptococcus pneumoniae*	This patent	*pbp2b*
1026	*Streptococcus pneumoniae*	This patent	*pbp2b*
1027	*Streptococcus pneumoniae*	This patent	*pbp2b*
1028	*Streptococcus pneumoniae*	This patent	*pbp2b*
1029	*Streptococcus pneumoniae*	This patent	*pbp2b*
1030	*Streptococcus pneumoniae*	This patent	*pbp2b*
1031	*Streptococcus pneumoniae*	This patent	*pbp2b*
1032	*Streptococcus pneumoniae*	This patent	*pbp2b*
1033	*Streptococcus pneumoniae*	This patent	*pbp2b*
1034	*Streptococcus pneumoniae*	This patent	*pbp2x*
1035	*Streptococcus pneumoniae*	This patent	*pbp2x*
1036	*Streptococcus pneumoniae*	This patent	*pbp2x*
1037	*Streptococcus pneumoniae*	This patent	*pbp2x*

Table 7. Origin of the nucleic acids and/or sequences in the sequence listing (continued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
1038	*Streptococcus pneumoniae*	This patent	*pbp2x*
1039	*Streptococcus pneumoniae*	This patent	*pbp2x*
1040	*Streptococcus pneumoniae*	This patent	*pbp2x*
1041	*Streptococcus pneumoniae*	This patent	*pbp2x*
1042	*Streptococcus pneumoniae*	This patent	*pbp2x*
1043	*Streptococcus pneumoniae*	This patent	*pbp2x*
1044	*Streptococcus pneumoniae*	This patent	*pbp2x*
1045	*Streptococcus pneumoniae*	This patent	*pbp2x*
1046	*Streptococcus pneumoniae*	This patent	*pbp2x*
1047	*Streptococcus pneumoniae*	This patent	*pbp2x*
1048	*Streptococcus pneumoniae*	This patent	*pbp2x*
1049	*Enterococcus faecium*	This patent	*vanA*
1050	*Enterococcus gallinarum*	This patent	*vanA*
1051	*Enterococcus faecium*	This patent	*vanA*
1052	*Enterococcus faecium*	This patent	*vanA*
1053	*Enterococcus faecium*	This patent	*vanA*
1054	*Enterococcus faecalis*	This patent	*vanA*
1055	*Enterococcus gallinarum*	This patent	*vanA*
1056	*Enterococcus faecium*	This patent	*vanA*
1057	*Enterococcus flavescens*	This patent	*vanA*
1058	*Enterococcus gallinarum*	This patent	*vanC1*
1059	*Enterococcus gallinarum*	This patent	*vanC1*
1060	*Enterococcus casseliflavus*	This patent	*vanC2*
1061	*Enterococcus casseliflavus*	This patent	*vanC2*
1062	*Enterococcus casseliflavus*	This patent	*vanC2*
1063	*Enterococcus casseliflavus*	This patent	*vanC2*
1064	*Enterococcus flavescens*	This patent	*vanC3*
1065	*Enterococcus flavescens*	This patent	*vanC3*
1066	*Enterococcus flavescens*	This patent	*vanC3*
1067	*Enterococcus faecium*	This patent	*vanXY*
1068	*Enterococcus faecium*	This patent	*vanXY*
1069	*Enterococcus faecium*	This patent	*vanXY*
1070	*Enterococcus faecalis*	This patent	*vanXY*
1071	*Enterococcus gallinarum*	This patent	*vanXY*
1072	*Enterococcus faecium*	This patent	*vanXY*
1073	*Enterococcus flavescens*	This patent	*vanXY*
1074	*Enterococcus faecium*	This patent	*vanXY*
1075	*Enterococcus gallinarum*	This patent	*vanXY*
1076	*Escherichia coli*	Database	stx_1
1077	*Escherichia coli*	Database	stx_2
1093	*Staphylococcus saprophyticus*	This patent	unknown
1117	*Enterococcus faecium*	Database	*vanB*
1138	*Enterococcus gallinarum*	Database	*vanC*1
1139	*Enterococcus faecium*	Database	*vanA*
1140	*Enterococcus casseliflavus*	Database	*vanC2*
1141	*Enterococcus faecium*	Database	*vanHAXY*
1169	*Streptococcus pneumoniae*	Database	*pbp1a*
1172	*Streptococcus pneumoniae*	Database	*pbp2b*
1173	*Streptococcus pneumoniae*	Database	*pbp2x*
1178	*Staphylococcus aureus*	Database	*mec*A
1183	*Streptococcus pneumoniae*	Database	*hexA*
1184	*Streptococcus pneumoniae*	This patent	*hexA*
1185	*Streptococcus pneumoniae*	This patent	*hexA*
1186	*Streptococcus pneumoniae*	This patent	*hexA*
1187	*Streptococcus pneumoniae*	This patent	*hexA*

Tabl 7. Origin of the nucl ic acids and/or sequences in th sequ nc listing (continued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
1188	*Streptococcus oralis*	This patent	*hexA*
1189	*Streptococcus mitis*	This patent	*hexA*
1190	*Streptococcus mitis*	This patent	*hexA*
1191	*Streptococcus mitis*	This patent	*hexA*
1198	*Staphylococcus saprophyticus*	This patent	unknown
1215	*Streptococcus pyogenes*	Database	*pcp*
1230	*Escherichia coli*	Database	*tuf* (EF-G)
1242	*Enterococcus faecium*	Database	*ddl*
1243	*Enterococcus faecalis*	Database	*mtlF, mtlD*
1244	*Staphylococcus aureus* subsp. *aureus*	This patent	unknown
1245	*Bacillus anthracis*	This patent	*atpD*
1246	*Bacillus mycoides*	This patent	*atpD*
1247	*Bacillus thuringiensis*	This patent	*atpD*
1248	*Bacillus thuringiensis*	This patent	*atpD*
1249	*Bacillus thuringiensis*	This patent	*atpD*
1250	*Bacillus weihenstephanensis*	This patent	*atpD*
1251	*Bacillus thuringiensis*	This patent	*atpD*
1252	*Bacillus thuringiensis*	This patent	*atpD*
1253	*Bacillus cereus*	This patent	*atpD*
1254	*Bacillus cereus*	This patent	*atpD*
1255	*Staphylococcus aureus*	This patent	*gyrA*
1256	*Bacillus weihenstephanensis*	This patent	*atpD*
1257	*Bacillus anthracis*	This patent	*atpD*
1258	*Bacillus thuringiensis*	This patent	*atpD*
1259	*Bacillus cereus*	This patent	*atpD*
1260	*Bacillus cereus*	This patent	*atpD*
1261	*Bacillus thuringiensis*	This patent	*atpD*
1262	*Bacillus thuringiensis*	This patent	*atpD*
1263	*Bacillus thuringiensis*	This patent	*atpD*
1264	*Bacillus thuringiensis*	This patent	*atpD*
1265	*Bacillus anthracis*	This patent	*atpD*
1266	*Paracoccidioides brasiliensis*	This patent	*tuf* (EF-1)
1267	*Blastomyces dermatitidis*	This patent	*tuf* (EF-1)
1268	*Histoplasma capsulatum*	This patent	*tuf* (EF-1)
1269	*Trichophyton rubrum*	This patent	*tuf* (EF-1)
1270	*Microsporum canis*	This patent	*tuf* (EF-1)
1271	*Aspergillus versicolor*	This patent	*tuf* (EF-1)
1272	*Exophiala moniliae*	This patent	*tuf* (EF-1)
1273	*Hortaea werneckii*	This patent	*tuf* (EF-1)
1274	*Fusarium solani*	This patent	*tuf* (EF-1)
1275	*Aureobasidium pullulans*	This patent	*tuf* (EF-1)
1276	*Blastomyces dermatitidis*	This patent	*tuf* (EF-1)
1277	*Exophiala dermatitidis*	This patent	*tuf* (EF-1)
1278	*Fusarium moniliforme*	This patent	*tuf* (EF-1)
1279	*Aspergillus terreus*	This patent	*tuf* (EF-1)
1280	*Aspergillus fumigatus*	This patent	*tuf* (EF-1)
1281	*Cryptococcus laurentii*	This patent	*tuf* (EF-1)
1282	*Emmonsia parva*	This patent	*tuf* (EF-1)
1283	*Fusarium solani*	This patent	*tuf* (EF-1)
1284	*Sporothrix schenckii*	This patent	*tuf* (EF-1)
1285	*Aspergillus nidulans*	This patent	*tuf* (EF-1)
1286	*Cladophialophora carrionii*	This patent	*tuf* (EF-1)
1287	*Exserohilum rostratum*	This patent	*tuf* (EF-1)
1288	*Bacillus thuringiensis*	This patent	*recA*
1289	*Bacillus thuringiensis*	This patent	*recA*
1299	*Staphylococcus aureus*	Databas	*gyrA*
1300	*Escherichia coli*	Database	*gyrA*
1307	*Staphylococcus aureus*	Database	*gyrB*
1320	*Escherichia coli*	Database	*parC (grlA)*
1321	*Staphylococcus aureus*	Database	*parC (grlA)*
1328	*Staphylococcus aureus*	Database	*parE (grlB)*

Table 7. Origin of th nucleic acids and/or s qu nces in th s qu nc listing (continued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
1348	unidentified bacterium	Database	*aac2Ia*
1351	*Pseudomonas aeruginosa*	Database	*aac3Ib*
1356	*Serratia marcescens*	Database	*aac3IIb*
1361	*Escherichia coli*	Database	*aac3IVa*
1366	*Enterobacter cloacae*	Database	*aac3VIa*
1371	*Citrobacter koseri*	Database	*aac6Ia*
1376	*Serratia marcescens*	Database	*aac6Ic*
1381	*Escherichia coli*	Database	*ant3Ia*
1386	*Staphylococcus aureus*	Database	*ant4Ia*
1391	*Escherichia coli*	Database	*aph3Ia*
1396	*Escherichia coli*	Database	*aph3IIa*
1401	*Enterococcus faecalis*	Database	*aph3IIIa*
1406	*Acinetobacter baumannii*	Database	*aph3VIa*
1411	*Pseudomonas aeruginosa*	Database	*blaCARB*
1416	*Klebsiella pneumoniae*	Database	*blaCMY-2*
1423	*Escherichia coli*	Database	*blaCTX-M-1*
1428	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Typhimurium	Database	*blaCTX-M-2*
1433	*Pseudomonas aeruginosa*	Database	*blaIMP*
1438	*Escherichia coli*	Database	*blaOXA2*
1439	*Pseudomonas aeruginosa*	Database	*blaOXA10*
1442	*Pseudomonas aeruginosa*	Database	*blaPER1*
1445	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Typhimurium	Database	*blaPER2*
1452	*Staphylococcus epidermidis*	Database	*dfrA*
1461	*Escherichia coli*	Database	*dhfrIa*
1470	*Escherichia coli*	Database	*dhfrIb*
1475	*Escherichia coli*	Database	*dhfrV*
1480	*Proteus mirabilis*	Database	*dhfrVI*
1489	*Escherichia coli*	Database	*dhfrVII*
1494	*Escherichia coli*	Database	*dhfrVIII*
1499	*Escherichia coli*	Database	*dhfrIX*
1504	*Escherichia coli*	Database	*dhfrXII*
1507	*Escherichia coli*	Database	*dhfrXIII*
1512	*Escherichia coli*	Database	*dhfrXV*
1517	*Escherichia coli*	Database	*dhfrXVII*
1518	*Acinetobacter lwoffii*	This patent	*fusA*
1519	*Acinetobacter lwoffii*	This patent	*fusA-tuf* spacer
1520	*Acinetobacter lwoffii*	This patent	*tuf*
1521	*Haemophilus influenzae*	This patent	*fusA*
1522	*Haemophilus influenzae*	This patent	*fusA-tuf* spacer
1523	*Haemophilus influenzae*	This patent	*tuf*
1524	*Proteus mirabilis*	This patent	*fusA*
1525	*Proteus mirabilis*	This patent	*fusA-tuf* spacer
1526	*Proteus mirabilis*	This patent	*tuf*
1527	*Campylobacter curvus*	This patent	*atpD*
1530	*Escherichia coli*	Database	*ereA*
1535	*Escherichia coli*	Database	*ereB*
1540	*Staphylococcus haemolyticus*	Database	*linA*
1545	*Enterococcus faecium*	Database	*linB*
1548	*Streptococcus pyogenes*	Database	*mefA*
1551	*Streptococcus pneumoniae*	Database	*mefE*
1560	*Escherichia coli*	Database	*mphA*
1561	*Candida albicans*	This patent	*tuf* (EF-1)
1562	*Candida dubliniensis*	This patent	*tuf* (EF-1)
1563	*Candida famata*	This patent	*tuf* (EF-1)
1564	*Candida glabrata*	This patent	*tuf* (EF-1)
1565	*Candida guilliermondii*	This patent	*tuf* (EF-1)
1566	*Candida haemulonii*	This patent	*tuf* (EF-1)
1567	*Candida kefyr*	This patent	*tuf* (EF-1)
1568	*Candida lusitaniae*	This patent	*tuf* (EF-1)

Tabl 7. Origin of the nucleic acids and/or s qu nc s in the sequ nce listing (continu d).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
1569	*Candida sphaerica*	This patent	*tuf* (EF-1)
1570	*Candida tropicalis*	This patent	*tuf* (EF-1)
1571	*Candida viswanathii*	This patent	*tuf* (EF-1)
1572	*Alcaligenes faecalis* subsp. *faecalis*	This patent	*tuf*
1573	*Prevotella buccalis*	This patent	*tuf*
1574	*Succinivibrio dextrinosolvens*	This patent	*tuf*
1575	*Tetragenococcus halophilus*	This patent	*tuf*
1576	*Campylobacter jejuni* subsp. *jejuni*	This patent	*atpD*
1577	*Campylobacter rectus*	This patent	*atpD*
1578	*Enterococcus casseliflavus*	This patent	*fusA*
1579	*Enterococcus gallinarum*	This patent	*fusA*
1580	*Streptococcus mitis*	This patent	*fusA*
1585	*Enterococcus faecium*	Database	*satG*
1590	Cloning vector pFW16	Database	*tetM*
1594	*Enterococcus faecium*	Database	*vanD*
1599	*Enterococcus faecalis*	Database	*vanE*
1600	*Campylobacter jejuni* subsp. *doylei*	This patent	*atpD*
1601	*Enterococcus sulfureus*	This patent	*atpD*
1602	*Enterococcus solitarius*	This patent	*atpD*
1603	*Campylobacter sputorum* subsp. *sputorum*	This patent	*atpD*
1604	*Enterococcus pseudoavium*	This patent	*atpD*
1607	*Klebsiella omithinolytica*	This patent	*gyrA*
1608	*Klebsiella oxytoca*	This patent	*gyrA*
1613	*Staphylococcus aureus*	Database	*vatB*
1618	*Staphylococcus cohnii*	Database	*vatC*
1623	*Staphylococcus aureus*	Database	*vga*
1628	*Staphylococcus aureus*	Database	*vgaB*
1633	*Staphylococcus aureus*	Database	*vgb*
1638	*Aspergillus fumigatus*	This patent	*atpD*
1639	*Aspergillus fumigatus*	This patent	*atpD*
1640	*Bacillus mycoides*	This patent	*atpD*
1641	*Bacillus mycoides*	This patent	*atpD*
1642	*Bacillus mycoides*	This patent	*atpD*
1643	*Bacillus pseudomycoides*	This patent	*atpD*
1644	*Bacillus pseudomycoides*	This patent	*atpD*
1645	*Budvicia aquatica*	This patent	*atpD*
1646	*Buttiauxella agrestis*	This patent	*atpD*
1647	*Candida norvegica*	This patent	*atpD*
1648	*Streptococcus pneumoniae*	This patent	*pbp1a*
1649	*Campylobacter lari*	This patent	*atpD*
1650	*Coccidioides immitis*	This patent	*atpD*
1651	*Emmonsia parva*	This patent	*atpD*
1652	*Erwinia amylovora*	This patent	*atpD*
1653	*Fonsecaea pedrosoi*	This patent	*atpD*
1654	*Fusarium moniliforme*	This patent	*atpD*
1655	*Klebsiella oxytoca*	This patent	*atpD*
1656	*Microsporum audouinii*	This patent	*atpD*
1657	*Obesumbacterium proteus*	This patent	*atpD*
1658	*Paracoccidioides brasiliensis*	This patent	*atpD*
1659	*Plesiomonas shigelloides*	This patent	*atpD*
1660	*Shewanella putrefaciens*	This patent	*atpD*
1662	*Campylobacter curvus*	This patent	*tuf*
1663	*Campylobacter rectus*	This patent	*tuf*
1664	*Fonsecaea pedrosoi*	This patent	*tuf*
1666	*Microsporum audouinii*	This patent	*tuf*
1667	*Piedraia hortai*	This patent	*tuf*
1668	*Escherichia coli*	Database	*tuf*
1669	*Saksenaea vasiformis*	This patent	*tuf*
1670	*Trichophyton tonsurans*	This pat nt	*tuf*
1671	*Enterobacter aerogenes*	This patent	*atpD*
1672	*Bordetella pertussis*	Databas	*atpD*
1673	*Arcanobacterium haemolyticum*	This pat nt	*tuf*

Tabl 7. Origin f th nucl ic acids and/ r s quenc s in th sequ nc listing (c ntinu d).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
1674	*Butyrivibrio fibrisolvens*	This patent	*tuf*
1675	*Campylobacter jejuni* subsp. *doylei*	This patent	*tuf*
1676	*Campylobacter lari*	This patent	*tuf*
1677	*Campylobacter sputorum* subsp. *sputorum*	This patent	*tuf*
1678	*Campylobacter upsaliensis*	This patent	*tuf*
1679	*Globicatella sanguis*	This patent	*tuf*
1680	*Lactobacillus acidophilus*	This patent	*tuf*
1681	*Leuconostoc mesenteroides* subsp. *dextranicum*	This patent	*tuf*
1682	*Prevotella buccalis*	This patent	*tuf*
1683	*Ruminococcus bromii*	This patent	*tuf*
1684	*Paracoccidioides brasiliensis*	This patent	*atpD*
1685	*Candida norvegica*	This patent	*tuf* (EF-1)
1686	*Aspergillus nidulans*	This patent	*tuf*
1687	*Aspergillus terreus*	This patent	*tuf*
1688	*Candida norvegica*	This patent	*tuf*
1689	*Candida parapsilosis*	This patent	*tuf*
1702	*Streptococcus gordonii*	WO98/20157	*recA*
1703	*Streptococcus mutans*	WO98/20157	*recA*
1704	*Streptococcus pneumoniae*	WO98/20157	*recA*
1705	*Streptococcus pyogenes*	WO98/20157	*recA*
1706	*Streptococcus salivarius* subsp. *thermophilus*	WO98/20157	*recA*
1707	*Escherichia coli*	WO98/20157	*oxa*
1708	*Enterococcus faecalis*	WO98/20157	*blaZ*
1709	*Pseudomonas aeruginosa*	WO98/20157	*aac6'-IIa*
1710	*Staphylococcus aureus*	WO98/20157	*ermA*
1711	*Escherichia coli*	WO98/20157	*ermB*
1712	*Staphylococcus aureus*	WO98/20157	*ermC*
1713	*Enterococcus faecalis*	WO98/20157	*vanB*
1714	*Campylobacter jejuni* subsp. *jejuni*	This patent	*recA*
1715	*Abiotrophia adiacens*	WO98/20157	*tuf*
1716	*Abiotrophia defectiva*	WO98/20157	*tuf*
1717	*Corynebacterium accolens*	WO98/20157	*tuf*
1718	*Corynebacterium genitalium*	WO98/20157	*tuf*
1719	*Corynebacterium jeikeium*	WO98/20157	*tuf*
1720	*Corynebacterium pseudodiphtheriticum*	WO98/20157	*tuf*
1721	*Corynebacterium striatum*	WO98/20157	*tuf*
1722	*Enterococcus avium*	WO98/20157	*tuf*
1723	*Gardnerella vaginalis*	WO98/20157	*tuf*
1724	*Listeria innocua*	WO98/20157	*tuf*
1725	*Listeria ivanovii*	WO98/20157	*tuf*
1726	*Listeria monocytogenes*	WO98/20157	*tuf*
1727	*Listeria seeligeri*	WO98/20157	*tuf*
1728	*Staphylococcus aureus*	WO98/20157	*tuf*
1729	*Staphylococcus saprophyticus*	WO98/20157	*tuf*
1730	*Staphylococcus simulans*	WO98/20157	*tuf*
1731	*Streptococcus agalactiae*	WO98/20157	*tuf*
1732	*Streptococcus pneumoniae*	WO98/20157	*tuf*
1733	*Streptococcus salivarius*	WO98/20157	*tuf*
1734	*Agrobacterium radiobacter*	WO98/20157	*tuf*
1735	*Bacillus subtilis*	WO98/20157	*tuf*
1736	*Bacteroides fragilis*	WO98/20157	*tuf*
1737	*Borrelia burgdorferi*	WO98/20157	*tuf*
1738	*Brevibacterium linens*	WO98/20157	*tuf*
1739	*Chlamydia trachomatis*	WO98/20157	*tuf*
1740	*Fibrobacter succinogenes*	WO98/20157	*tuf*
1741	*Flavobacterium ferrugineum*	WO98/20157	*tuf*
1742	*Helicobacter pylori*	WO98/20157	*tuf*
1743	*Micrococcus luteus*	WO98/20157	*tuf*
1744	*Mycobacterium tuberculosis*	WO98/20157	*tuf*
1745	*Mycoplasma genitalium*	WO98/20157	*tuf*
1746	*Neisseria gonorrhoeae*	WO98/20157	*tuf*

Table 7. Origin f th nucleic acids and/or s qu nces in the s quence listing (continued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
1747	*Rickettsia prowazekii*	WO98/20157	*tuf*
1748	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Typhimurium	WO98/20157	*tuf*
1749	*Shewanella putrefaciens*	WO98/20157	*tuf*
1750	*Stigmatella aurantiaca*	WO98/20157	*tuf*
1751	*Thiomonas cuprina*	WO98/20157	*tuf*
1752	*Treponema pallidum*	WO98/20157	*tuf*
1753	*Ureaplasma urealyticum*	WO98/20157	*tuf*
1754	*Wolinella succinogenes*	WO98/20157	*tuf*
1755	*Burkholderia cepacia*	WO98/20157	*tuf*
1756	*Bacillus anthracis*	This patent	*recA*
1757	*Bacillus anthracis*	This patent	*recA*
1758	*Bacillus cereus*	This patent	*recA*
1759	*Bacillus cereus*	This patent	*recA*
1760	*Bacillus mycoides*	This patent	*recA*
1761	*Bacillus pseudomycoides*	This patent	*recA*
1762	*Bacillus thuringiensis*	This patent	*recA*
1763	*Bacillus thuringiensis*	This patent	*recA*
1764	*Klebsiella oxytoca*	This patent	*gyrA*
1765	*Klebsiella pneumoniae* subsp. *ozaenae*	This patent	*gyrA*
1766	*Klebsiella planticola*	This patent	*gyrA*
1767	*Klebsiella pneumoniae*	This patent	*gyrA*
1768	*Klebsiella pneumoniae* subsp. *pneumoniae*	This patent	*gyrA*
1769	*Klebsiella pneumoniae* subsp. *pneumoniae*	This patent	*gyrA*
1770	*Klebsiella pneumoniae* subsp. *rhinoscleromatis*	This patent	*gyrA*
1771	*Klebsiella terrigena*	This patent	*gyrA*
1772	*Legionella pneumophila* subsp. *pneumophila*	This patent	*gyrA*
1773	*Proteus mirabilis*	This patent	*gyrA*
1774	*Providencia rettgeri*	This patent	*gyrA*
1775	*Proteus vulgaris*	This patent	*gyrA*
1776	*Yersinia enterocolitica*	This patent	*gyrA*
1777	*Klebsiella oxytoca*	This patent	*parC (grlA)*
1778	*Klebsiella oxytoca*	This patent	*parC (grlA)*
1779	*Klebsiella pneumoniae* subsp. *ozaenae*	This patent	*parC (grlA)*
1780	*Klebsiella planticola*	This patent	*parC (grlA)*
1781	*Klebsiella pneumoniae*	This patent	*parC (grlA)*
1782	*Klebsiella pneumoniae* subsp. *pneumoniae*	This patent	*parC (grlA)*
1783	*Klebsiella pneumoniae* subsp. *pneumoniae*	This patent	*parC (grlA)*
1784	*Klebsiella pneumoniae* subsp. *rhinoscleromatis*	This patent	*parC (grlA)*
1785	*Klebsiella terrigena*	This patent	*parC (grlA)*
1786	*Bacillus cereus*	This patent	*fusA*
1787	*Bacillus cereus*	This patent	*fusA*
1788	*Bacillus anthracis*	This patent	*fusA*
1789	*Bacillus cereus*	This patent	*fusA*
1790	*Bacillus anthracis*	This patent	*fusA*
1791	*Bacillus pseudomycoides*	This patent	*fusA*
1792	*Bacillus cereus*	This patent	*fusA*
1793	*Bacillus anthracis*	This patent	*fusA*
1794	*Bacillus cereus*	This patent	*fusA*
1795	*Bacillus weihenstephanensis*	This patent	*fusA*
1796	*Bacillus mycoides*	This patent	*fusA*
1797	*Bacillus thuringiensis*	This patent	*fusA*
1798	*Bacillus weihenstephanensis*	This patent	*fusA-tuf* spacer
1799	*Bacillus thuringiensis*	This patent	*fusA-tuf* spacer
1800	*Bacillus anthracis*	This patent	*fusA-tuf* spacer
1801	*Bacillus pseudomycoides*	This patent	*fusA-tuf* spacer
1802	*Bacillus anthracis*	This patent	*fusA-tuf* spacer
1803	*Bacillus cereus*	This patent	*fusA-tuf* spacer
1804	*Bacillus cereus*	This patent	*fusA-tuf* spacer
1805	*Bacillus mycoides*	This patent	*fusA-tuf* spacer
1806	*Bacillus cereus*	This patent	*fusA-tuf* spacer

Table 7. Origin of the nucl ic acids and/ r s quenc s in the sequ nc listing (continued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
1807	*Bacillus cereus*	This patent	*fusA-tuf* spacer
1808	*Bacillus cereus*	This patent	*fusA-tuf* spacer
1809	*Bacillus anthracis*	This patent	*fusA-tuf* spacer
1810	*Bacillus mycoides*	This patent	*tuf*
1811	*Bacillus thuringiensis*	This patent	*tuf*
1812	*Bacillus cereus*	This patent	*tuf*
1813	*Bacillus weihenstephanensis*	This patent	*tuf*
1814	*Bacillus anthracis*	This patent	*tuf*
1815	*Bacillus cereus*	This patent	*tuf*
1816	*Bacillus cereus*	This patent	*tuf*
1817	*Bacillus anthracis*	This patent	*tuf*
1818	*Bacillus cereus*	This patent	*tuf*
1819	*Bacillus anthracis*	This patent	*tuf*
1820	*Bacillus pseudomycoides*	This patent	*tuf*
1821	*Bacillus cereus*	This patent	*tuf*
1822	*Streptococcus oralis*	This patent	*fusA*
1823	*Budvicia aquatica*	This patent	*fusA*
1824	*Buttiauxella agrestis*	This patent	*fusA*
1825	*Klebsiella oxytoca*	This patent	*fusA*
1826	*Plesiomonas shigelloides*	This patent	*fusA*
1827	*Shewanella putrefaciens*	This patent	*fusA*
1828	*Obesumbacterium proteus*	This patent	*fusA*
1829	*Klebsiella oxytoca*	This patent	*fusA-tuf* spacer
1830	*Budvicia aquatica*	This patent	*fusA-tuf* spacer
1831	*Plesiomonas shigelloides*	This patent	*fusA-tuf* spacer
1832	*Obesumbacterium proteus*	This patent	*fusA-tuf* spacer
1833	*Shewanella putrefaciens*	This patent	*fusA-tuf* spacer
1834	*Buttiauxella agrestis*	This patent	*fusA-tuf* spacer
1835	*Campylobacter coli*	This patent	*tuf*
1836	*Campylobacter fetus* subsp. *fetus*	This patent	*tuf*
1837	*Campylobacter fetus* subsp. *venerealis*	This patent	*tuf*
1838	*Buttiauxella agrestis*	This patent	*tuf*
1839	*Klebsiella oxytoca*	This patent	*tuf*
1840	*Plesiomonas shigelloides*	This patent	*tuf*
1841	*Shewanella putrefaciens*	This patent	*tuf*
1842	*Obesumbacterium proteus*	This patent	*tuf*
1843	*Budvicia aquatica*	This patent	*tuf*
1844	*Abiotrophia adiacens*	This patent	*atpD*
1845	*Arcanobacterium haemolyticum*	This patent	*atpD*
1846	*Basidiobolus ranarum*	This patent	*atpD*
1847	*Blastomyces dermatitidis*	This patent	*atpD*
1848	*Blastomyces dermatitidis*	This patent	*atpD*
1849	*Campylobacter coli*	This patent	*atpD*
1850	*Campylobacter fetus* subsp. *fetus*	This patent	*atpD*
1851	*Campylobacter fetus* subsp. *venerealis*	This patent	*atpD*
1852	*Campylobacter gracilis*	This patent	*atpD*
1853	*Campylobacter jejuni* subsp. *jejuni*	This patent	*atpD*
1854	*Enterococcus cecorum*	This patent	*atpD*
1855	*Enterococcus columbae*	This patent	*atpD*
1856	*Enterococcus dispar*	This patent	*atpD*
1857	*Enterococcus malodoratus*	This patent	*atpD*
1858	*Enterococcus mundtii*	This patent	*atpD*
1859	*Enterococcus raffinosus*	This patent	*atpD*
1860	*Globicatella sanguis*	This patent	*atpD*
1861	*Lactococcus garvieae*	This patent	*atpD*
1862	*Lactococcus lactis*	This patent	*atpD*
1863	*Listeria ivanovii*	This patent	*atpD*
1864	*Succinivibrio dextrinosolvens*	This patent	*atpD*
1865	*Tetragenococcus halophilus*	This patent	*atpD*
1866	*Campylobacter fetus* subsp. *fetus*	This patent	*recA*
1867	*Campylobacter fetus* subsp. *venerealis*	This patent	*recA*
1868	*Campylobacter jejuni* subsp. *jejuni*	This patent	*recA*

Tabl 7. Origin of the nucl ic acids and/or sequenc s in th sequ nce listing (continued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
1869	*Enterococcus avium*	This patent	*recA*
1870	*Enterococcus faecium*	This patent	*recA*
1871	*Listeria monocytogenes*	This patent	*recA*
1872	*Streptococcus mitis*	This patent	*recA*
1873	*Streptococcus oralis*	This patent	*recA*
1874	*Aspergillus fumigatus*	This patent	*tuf* (M)
1875	*Aspergillus versicolor*	This patent	*tuf* (M)
1876	*Basidiobolus ranarum*	This patent	*tuf* (M)
1877	*Campylobacter gracilis*	This patent	*tuf*
1878	*Campylobacter jejuni* subsp. *jejuni*	This patent	*tuf*
1879	*Coccidioides immitis*	This patent	*tuf* (M)
1880	*Erwinia amylovora*	This patent	*tuf*
1881	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Typhimurium	This patent	*tuf*
1899	*Klebsiella pneumoniae*	Database	*blaSHV*
1900	*Klebsiella pneumoniae*	Database	*blaSHV*
1901	*Escherichia coli*	Database	*blaSHV*
1902	*Klebsiella pneumoniae*	Database	*blaSHV*
1903	*Klebsiella pneumoniae*	Database	*blaSHV*
1904	*Escherichia coli*	Database	*blaSHV*
1905	*Pseudomonas aeruginosa*	Database	*blaSHV*
1927	*Neisseria meningitidis*	Database	*blaTEM*
1928	*Escherichia coli*	Database	*blaTEM*
1929	*Klebsiella oxytoca*	Database	*blaTEM*
1930	*Escherichia coli*	Database	*blaTEM*
1931	*Escherichia coli*	Database	*blaTEM*
1932	*Escherichia coli*	Database	*blaTEM*
1933	*Escherichia coli*	Database	*blaTEM*
1954	*Klebsiella pneumoniae* subsp. *pneumoniae*	Database	*gyrA*
1956	*Candida inconspicua*	This patent	*tuf* (M)
1957	*Candida utilis*	This patent	*tuf* (M)
1958	*Candida zeylanoides*	This patent	*tuf* (M)
1959	*Candida catenulata*	This patent	*tuf* (M)
1960	*Candida krusei*	This patent	*tuf* (M)
1965	Plasmid pGS05	Database	*sulII*
1970	Transposon Tn10	Database	*tetB*
1985	*Cryptococcus neoformans*	Database	*tuf* (EF-1)
1986	*Cryptococcus neoformans*	Database	*tuf* (EF-1)
1987	*Saccharomyces cerevisiae*	Database	*tuf* (EF-1)
1988	*Saccharomyces cerevisiae*	Database	*tuf* (EF-1)
1989	*Eremothecium gossypii*	Database	*tuf* (EF-1)
1990	*Eremothecium gossypii*	Database	*tuf* (EF-1)
1991	*Aspergillus oryzae*	Database	*tuf* (EF-1)
1992	*Aureobasidium pullulans*	Database	*tuf* (EF-1)
1993	*Histoplasma capsulatum*	Database	*tuf* (EF-1)
1994	*Neurospora crassa*	Database	*tuf* (EF-1)
1995	*Podospora anserina*	Database	*tuf* (EF-1)
1996	*Podospora curvicolla*	Database	*tuf* (EF-1)
1997	*Sordaria macrospora*	Database	*tuf* (EF-1)
1998	*Trichoderma reesei*	Database	*tuf* (EF-1)
2004	*Candida albicans*	Database	*tuf* (M)
2005	*Schizosaccharomyces pombe*	Database	*tuf* (M)
2010	*Klebsiella pneumoniae*	Database	*blaTEM*
2011	*Klebsiella pneumoniae*	Database	*blaTEM*
2013	*Kluyvera ascorbata*	This patent	*gyrA*
2014	*Kluyvera georgiana*	This patent	*gyrA*
2047	*Streptococcus pneumoniae*	Database	*pbp1A*
2048	*Streptococcus pneumoniae*	Databas	*pbp1A*
2049	*Streptococcus pneumoniae*	Database	*pbp1A*

Table 7. Origin of th nucl ic acids and/or sequ nc s in the sequence listing (c ntinued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	G ne*
2050	*Streptococcus pneumoniae*	Database	*pbp1A*
2051	*Streptococcus pneumoniae*	Database	*pbp1A*
2052	*Streptococcus pneumoniae*	Database	*pbp1A*
2053	*Streptococcus pneumoniae*	Database	*pbp1A*
2054	*Streptococcus pneumoniae*	Database	*gyrA*
2055	*Streptococcus pneumoniae*	Database	*parC*
2056	*Streptococcus pneumoniae*	This patent	*pbp1A*
2057	*Streptococcus pneumoniae*	This patent	*pbp1A*
2058	*Streptococcus pneumoniae*	This patent	*pbp1A*
2059	*Streptococcus pneumoniae*	This patent	*pbp1A*
2060	*Streptococcus pneumoniae*	This patent	*pbp1A*
2061	*Streptococcus pneumoniae*	This patent	*pbp1A*
2062	*Streptococcus pneumoniae*	This patent	*pbp1A*
2063	*Streptococcus pneumoniae*	This patent	*pbp1A*
2064	*Streptococcus pneumoniae*	This patent	*pbp1A*
2072	*Mycobacterium tuberculosis*	Database	*rpoB*
2097	*Mycoplasma pneumoniae*	Database	*tuf*
2101	*Mycobacterium tuberculosis*	Database	*inhA*
2105	*Mycobacterium tuberculosis*	Database	*embB*
2129	*Clostridium difficile*	Database	*cdtA*
2130	*Clostridium difficile*	Database	*cdtB*
2137	*Pseudomonas putida*	Genome project	*tuf*
2138	*Pseudomonas aeruginosa*	Genome project	*tuf*
2139	*Campylobacter jejuni*	Database	*atpD*
2140	*Streptococcus pneumoniae*	Database	*pbp1a*
2144	*Staphylococcus aureus*	Database	*mupA*
2147	*Escherichia coli*	Database	*catI*
2150	*Escherichia coli*	Database	*catII*
2153	*Shigella flexneri*	Database	*catIII*
2156	*Clostridium perfringens*	Database	*catP*
2159	*Staphylococcus aureus*	Database	*cat*
2162	*Staphylococcus aureus*	Database	*cat*
2165	*Salmonella typhimurium*	Database	*ppflo*-like
2183	*Alcaligenes faecalis* subsp. *faecalis*	This patent	*tuf*
2184	*Campylobacter coli*	This patent	*fusA*
2185	*Succinivibrio dextrinosolvens*	This patent	*tuf*
2186	*Tetragenococcus halophilus*	This patent	*tuf*
2187	*Campylobacter jejuni* subsp. *jejuni*	This patent	*fusA*
2188	*Campylobacter jejuni* subsp. *jejuni*	This patent	*fusA*
2189	*Leishmania guyanensis*	This patent	*atpD*
2190	*Trypanosoma brucei brucei*	This patent	*atpD*
2191	*Aspergillus nidulans*	This patent	*atpD*
2192	*Leishmania panamensis*	This patent	*atpD*
2193	*Aspergillus nidulans*	This patent	*tuf* (M)
2194	*Aureobasidium pullulans*	This patent	*tuf* (M)
2195	*Emmonsia parva*	This patent	*tuf* (M)
2196	*Exserohilum rostratum*	This patent	*tuf* (M)
2197	*Fusarium moniliforme*	This patent	*tuf* (M)
2198	*Fusarium solani*	This patent	*tuf* (M)
2199	*Histoplasma capsulatum*	This patent	*tuf* (M)
2200	*Kocuria kristinae*	This patent	*tuf*
2201	*Vibrio mimicus*	This patent	*tuf*
2202	*Citrobacter freundii*	This patent	*recA*
2203	*Clostridium botulinum*	This patent	*recA*
2204	*Francisella tularensis*	This patent	*recA*
2205	*Peptostreptococcus anaerobius*	This patent	*recA*
2206	*Peptostreptococcus asaccharolyticus*	This patent	*recA*
2207	*Providencia stuartii*	This patent	*recA*

Table 7. Origin of th nucleic acids and/or sequenc s in the s quenc listing (continued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
2208	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Paratyphi A	This patent	*recA*
2209	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Typhimurium	This patent	*recA*
2210	*Staphylococcus saprophyticus*	This patent	*recA*
2211	*Yersinia pseudotuberculosis*	This patent	*recA*
2212	*Zoogloea ramigera*	This patent	*recA*
2214	*Abiotrophia adiacens*	This patent	*fusA*
2215	*Acinetobacter baumannii*	This patent	*fusA*
2216	*Actinomyces meyeri*	This patent	*fusA*
2217	*Clostridium difficile*	This patent	*fusA*
2218	*Corynebacterium diphtheriae*	This patent	*fusA*
2219	*Enterobacter cloacae*	This patent	*fusA*
2220	*Klebsiella pneumoniae* subsp. *pneumoniae*	This patent	*fusA*
2221	*Listeria monocytogenes*	This patent	*fusA*
2222	*Mycobacterium avium*	This patent	*fusA*
2223	*Mycobacterium gordonae*	This patent	*fusA*
2224	*Mycobacterium kansasii*	This patent	*fusA*
2225	*Mycobacterium terrae*	This patent	*fusA*
2226	*Neisseria polysaccharea*	This patent	*fusA*
2227	*Staphylococcus epidermidis*	This patent	*fusA*
2228	*Staphylococcus haemolyticus*	This patent	*fusA*
2229	*Succinivibrio dextrinosolvens*	This patent	*fusA*
2230	*Tetragenococcus halophilus*	This patent	*fusA*
2231	*Veillonella parvula*	This patent	*fusA*
2232	*Yersinia pseudotuberculosis*	This patent	*fusA*
2233	*Zoogloea ramigera*	This patent	*fusA*
2234	*Aeromonas hydrophila*	This patent	*fusA*
2235	*Abiotrophia adiacens*	This patent	*fusA-tuf* spacer
2236	*Acinetobacter baumannii*	This patent	*fusA-tuf* spacer
2237	*Actinomyces meyeri*	This patent	*fusA-tuf* spacer
2238	*Clostridium difficile*	This patent	*fusA-tuf* spacer
2239	*Corynebacterium diphtheriae*	This patent	*fusA-tuf* spacer
2240	*Enterobacter cloacae*	This patent	*fusA-tuf* spacer
2241	*Klebsiella pneumoniae* subsp. *pneumoniae*	This patent	*fusA-tuf* spacer
2242	*Listeria monocytogenes*	This patent	*fusA-tuf* spacer
2243	*Mycobacterium avium*	This patent	*fusA-tuf* spacer
2244	*Mycobacterium gordonae*	This patent	*fusA-tuf* spacer
2245	*Mycobacterium kansasii*	This patent	*fusA-tuf* spacer
2246	*Mycobacterium terrae*	This patent	*fusA-tuf* spacer
2247	*Neisseria polysaccharea*	This patent	*fusA-tuf* spacer
2248	*Staphylococcus epidermidis*	This patent	*fusA-tuf* spacer
2249	*Staphylococcus haemolyticus*	This patent	*fusA-tuf* spacer
2255	*Abiotrophia adiacens*	This patent	*tuf*
2256	*Acinetobacter baumannii*	This patent	*tuf*
2257	*Actinomyces meyeri*	This patent	*tuf*
2258	*Clostridium difficile*	This patent	*tuf*
2259	*Corynebacterium diphtheriae*	This patent	*tuf*
2260	*Enterobacter cloacae*	This patent	*tuf*
2261	*Klebsiella pneumoniae* subsp. *pneumoniae*	This patent	*tuf*
2262	*Listeria monocytogenes*	This patent	*tuf*
2263	*Mycobacterium avium*	This patent	*tuf*
2264	*Mycobacterium gordonae*	This patent	*tuf*
2265	*Mycobacterium kansasii*	This patent	*tuf*
2266	*Mycobacterium terrae*	This patent	*tuf*
2267	*Neisseria polysaccharea*	This pat nt	*tuf*
2268	*Staphylococcus epidermidis*	This patent	*tuf*
2269	*Staphylococcus haemolyticus*	This patent	*tuf*
2270	*Aeromonas hydrophila*	This pat nt	*tuf*
2271	*Bilophila wadsworthia*	This patent	*tuf*
2272	*Brevundimonas diminuta*	This patent	*tuf*
2273	*Streptococcus mitis*	This patent	*pbp1a*

Table 7. Origin of the nucleic acids and/or s quences in th sequ nce listing (continu d).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	G ne*
2274	*Streptococcus mitis*	This patent	*pbp1a*
2275	*Streptococcus mitis*	This patent	*pbp1a*
2276	*Streptococcus oralis*	This patent	*pbp1a*
2277	*Escherichia coli*	This patent	*gyrA*
2278	*Escherichia coli*	This patent	*gyrA*
2279	*Escherichia coli*	This patent	*gyrA*
2280	*Escherichia coli*	This patent	*gyrA*
2288	*Enterococcus faecium*	Database	*ddl*
2293	*Enterococcus faecium*	Database	*vanA*
2296	*Enterococcus faecalis*	Database	*vanB*

* *tuf* indicates *tuf* sequences, *tuf* (C) indicates *tuf* sequences divergent from main (usually A and B) copies of the elongation factor-Tu, *tuf* (EF-1) indicates *tuf* sequences of the eukaryotic type (elongation factor 1α), *tuf* (M) indicates *tuf* sequences from organellar (mostly mitochondrial) origin.

fusA indicates *fusA* sequences; *fusA-tuf* spacer indicates the intergenic region between *fusA* and *tuf*.

atpD indicates *atpD* sequences ot the F-type, *atpD* (V) indicates *atpD* sequences of the V-type.

recA indicates *recA* sequences, *recA*(Rad51) indicates *rad51* sequences or homologs and *recA*(Dmc1) indicates *dmc1* sequences or homologs.

Table 8. Bacterial sp cies us d to test th specificity of the *Streptococcus agalactiae*-sp cific amplificati n primers d rived fr m *tuf* sequ nces.

Strain	Reference number	Strain	Reference number
Streptococcus acidominimus	ATCC 51726	*Bacteroides caccae*	ATCC 43185
Streptococcus agalactiae	ATCC 12403	*Bacteroides vulgatus*	ATCC 8482
Streptococcus agalactiae	ATCC 12973	*Bacteroides fragilis*	ATCC 25285
Streptococcus agalactiae	ATCC 13813	*Candida albicans*	ATCC 11006
Streptococcus agalactiae	ATCC 27591	*Clostridium innoculum*	ATCC 14501
Streptococcus agalactiae	CDCs 1073	*Clostridium ramosum*	ATCC 25582
Streptococcus anginosus	ATCC 27335	*Lactobacillus casei* subsp. *casei*	ATCC 393
Streptococcus anginosus	ATCC 33397	*Clostridium septicum*	ATCC 12464
Streptococcus bovis	ATCC 33317	*Corynebacterium cervicis*	NCTC 10604
Streptococcus anginosus	ATCC 27823	*Corynebacterium genitalium*	ATCC 33031
Streptococcus cricetus	ATCC 19642	*Corynebacterium urealyticum*	ATCC 43042
Streptococcus cristatus	ATCC 51100	*Enterococcus faecalis*	ATCC 29212
Streptococcus downei	ATCC 33748	*Enterococcus faecium*	ATCC 19434
Streptococcus dysgalactiae	ATCC 43078	*Eubacterium lentum*	ATCC 43055
Streptococcus equi subsp. *equi*	ATCC 9528	*Eubacterium nodutum*	ATCC 33099
Streptococcus ferus	ATCC 33477	*Gardnerella vaginalis*	ATCC 14018
Streptococcus gordonii	ATCC 10558	*Lactobacillus acidophilus*	ATCC 4356
Streptococcus macacae	ATCC 35911	*Lactobacillus crispatus*	ATCC 33820
Streptococcus mitis	ATCC 49456	*Lactobacillus gasseri*	ATCC 33323
Streptococcus mutans	ATCC 25175	*Lactobacillus johnsonii*	ATCC 33200
Streptococcus oralis	ATCC 35037	*Lactococcus lactis* subsp. *lactis*	ATCC 19435
Streptococcus parasanguinis	ATCC 15912	*Lactococcus lactis* subsp. *lactis*	ATCC 11454
Streptococcus parauberis	DSM 6631	*Listeria innocua*	ATCC 33090
Streptococcus pneumoniae	ATCC 27336	*Micrococcus luteus*	ATCC 9341
Streptococcus pyogenes	ATCC 19615	*Escherichia coli*	ATCC 25922
Streptococcus ratti	ATCC 19645	*Micrococcus lylae*	ATCC 27566
Streptococcus salivarius	ATCC 7073	*Porphyromonas asaccharolytica*	ATCC 25260
Streptococcus sanguinis	ATCC 10556	*Prevotella corporis*	ATCC 33547
Streptococcus sobrinus	ATCC 27352	*Prevotella melanogenica*	ATCC 25845
Streptococcus suis	ATCC 43765	*Staphylococcus aureus*	ATCC 13301
Streptococcus uberis	ATCC 19436	*Staphylococcus epidermidis*	ATCC 14990
Streptococcus vestubularis	ATCC 49124	*Staphylococcus saprophyticus*	ATCC 15305

Table 9. Bact rial speci s used to test the specificity of the *Streptococcus agalactiae*-sp cific amplification primers derived fr m *atpD* sequ nces.

Strain	Reference number	Strain	Reference number
Streptococcus acidominimus	ATCC 51726	*Streptococcus gordonii*	ATCC 10558
Streptococcus agalactiae	ATCC 12400	*Streptococcus macacae*	ATCC 35911
Streptococcus agalactiae	ATCC 12403	*Streptococcus mitis*	ATCC 49456
Streptococcus agalactiae	ATCC 12973	*Streptococcus mutans*	ATCC 25175
Streptococcus agalactiae	ATCC 13813	*Streptococcus oralis*	ATCC 35037
Streptococcus agalactiae	ATCC 27591	*Streptococcus parasanguinis*	ATCC 15912
Streptococcus agalactiae	CDCs-1073	*Streptococcus parauberis*	DSM 6631
Streptococcus anginosus	ATCC 27335	*Streptococcus pneumoniae*	ATCC 27336
Streptococcus anginosus	ATCC 27823	*Streptococcus pyogenes*	ATCC 19615
Streptococcus bovis	ATCC 33317	*Streptococcus ratti*	ATCC 19645
Streptococcus cricetus	ATCC 19642	*Streptococcus salivarius*	ATCC 7073
Streptococcus cristatus	ATCC 51100	*Streptococcus sanguinis*	ATCC 10556
Streptococcus downei	ATCC 33748	*Streptococcus sobrinus*	ATCC 27352
Streptococcus dysgalactiae	ATCC 43078	*Streptococcus suis*	ATCC 43765
Streptococcus equi subsp. *equi*	ATCC 9528	*Streptococcus uberis*	ATCC 19436
Streptococcus ferus	ATCC 33477	*Streptococcus vestibularis*	ATCC 49124

Table 10. Bact rial sp cies used to test the specificity of the *Enterococcus*-sp cific amplificati n primers d rived fr m *tuf* sequenc s.

Strain	Reference number	Strain	Reference number
Gram-positive species (n=74)			
Abiotrophia adiacens	ATCC 49176	*Listeria innocua*	ATCC 33090
Abiotrophia defectiva	ATCC 49175	*Listeria ivanovii*	ATCC 19119
Bacillus cereus	ATCC 14579	*Listeria monocytogenes*	ATCC 15313
Bacillus subtilis	ATCC 27370	*Listeria seeligeri*	ATCC 35967
Bifidobacterium adolescentis	ATCC 27534	*Micrococcus luteus*	ATCC 9341
Bifidobacterium breve	ATCC 15700	*Pediococcus acidilacti*	ATCC 33314
Bifidobacterium dentium	ATCC 27534	*Pediococcus pentosaceus*	ATCC 33316
Bifidobacterium longum	ATCC 15707	*Peptococcus niger*	ATCC 27731
Clostridium perfringens	ATCC 3124	*Peptostreptococcus anaerobius*	ATCC 27337
Clostridium septicum	ATCC 12464	*Peptostreptococcus indolicus*	ATCC 29247
Corynebacterium aquaticus	ATCC 14665	*Peptostreptococcus micros*	ATCC 33270
Corynebacterium pseudodiphtheriticum	ATCC 10700	*Propionibacterium acnes*	ATCC 6919
		Staphylococcus aureus	ATCC 43300
Enterococcus avium	ATCC 14025	*Staphylococcus capitis*	ATCC 27840
Enterococcus casseliflavus	ATCC 25788	*Staphylococcus epidermidis*	ATCC 14990
Enterococcus cecorum	ATCC 43199	*Staphylococcus haemolyticus*	ATCC 29970
Enterococcus columbae	ATCC 51263	*Staphylococcus hominis*	ATCC 27844
Enterococcus dispar	ATCC 51266	*Staphylococcus lugdunensis*	ATCC 43809
Enterococcus durans	ATCC 19432	*Staphylococcus saprophyticus*	ATCC 15305
Enterococcus faecalis	ATCC 29212	*Staphylococcus simulans*	ATCC 27848
Enterococcus faecium	ATCC 19434	*Staphylococcus warneri*	ATCC 27836
Enterococcus flavescens	ATCC 49996	*Streptococcus agalactiae*	ATCC 13813
Enterococcus gallinarum	ATCC 49573	*Streptococcus anginosus*	ATCC 33397
Enterococcus hirae	ATCC 8044	*Streptococcus bovis*	ATCC 33317
Enterococcus malodoratus	ATCC 43197	*Streptococcus constellatus*	ATCC 27823
Enterococcus mundtii	ATCC 43186	*Streptococcus cristatus*	ATCC 51100
Enterococcus pseudoavium	ATCC 49372	*Streptococcus intermedius*	ATCC 27335
Enterococcus raffinosus	ATCC 49427	*Streptococcus mitis*	ATCC 49456
Enterococcus saccharolyticus	ATCC 43076	*Streptococcus mitis*	ATCC 3639
Enterococcus solitarius	ATCC 49428	*Streptococcus mutans*	ATCC 27175
Enterococcus sulfureus	ATCC 49903	*Streptococcus parasanguinis*	ATCC 15912
Eubacterium lentum	ATCC 49903	*Streptococcus pneumoniae*	ATCC 27736
Gemella haemolysans	ATCC 10379	*Streptococcus pneumoniae*	ATCC 6303
Gemella morbillorum	ATCC 27842	*Streptococcus pyogenes*	ATCC 19615
Lactobacillus acidophilus	ATCC 4356	*Streptococcus salivarius*	ATCC 7073
Leuconostoc mesenteroides	ATCC 19225	*Streptococcus sanguinis*	ATCC 10556
Listeria grayi	ATCC 19120	*Streptococcus suis*	ATCC 43765
Listeria grayi	ATCC 19123		

Table 10. Bact rial speci s us d to t st the specificity of th *Enterococcus*-sp cific amplificati n prim rs d rived fr m *tuf* sequenc s (c ntinued).

Strain	Reference number	Strain	Reference number
Gram-negative species (n=39)			
Acidominococcus fermentans	ATCC 2508	*Hafnia alvei*	ATCC 13337
Acinetobacter baumannii	ATCC 19606	*Klebsiella oxytoca*	ATCC 13182
Alcaligenes faecalis	ATCC 8750	*Meganomonas hypermegas*	ATCC 25560
Anaerobiospirillum succiniproducens	ATCC 29305	*Mitsukoella multiacidus*	ATCC 27723
		Moraxella catarrhalis	ATCC 43628
Anaerorhabdus furcosus	ATCC 25662	*Morganella morganii*	ATCC 25830
Bacteroides distasonis	ATCC 8503	*Neisseria meningitidis*	ATCC 13077
Bacteroides thetaiotaomicron	ATCC 29741	*Pasteurella aerogenes*	ATCC 27883
Bacteroides vulgatus	ATCC 8482	*Proteus vulgaris*	ATCC 13315
Bordetella pertussis	LSPQ 3702	*Providencia alcalifaciens*	ATCC 9886
Bulkholderia cepacia	LSPQ 2217	*Providencia rettgeri*	ATCC 9250
Butyvibrio fibrinosolvens	ATCC 19171	*Pseudomonas aeruginosa*	ATCC 27853
Cardiobacterium hominis	ATCC 15826	*Salmonella typhimurium*	ATCC 14028
Citrobacter freundii	ATCC 8090	*Serratia marcescens*	ATCC 13880
Desulfovibrio vulgaris	ATCC 29579	*Shigella flexneri*	ATCC 12022
Edwardsiellae tarda	ATCC 15947	*Shigella sonnei*	ATCC 29930
Enterobacter cloacae	ATCC 13047	*Succinivibrio dextrinosolvens*	ATCC 19716
Escherichia coli	ATCC 25922	*Tissierella praeacuta*	ATCC 25539
Fusobacterium russii	ATCC 25533	*Veillonella parvula*	ATCC 10790
Haemophilus influenzae	ATCC 9007	*Yersinia enterocolitica*	ATCC 9610

Table 11. Microbial species for which *tuf* and/ r *atpD* and/ r *recA* s qu nces are available in public databases.

Species	Strain	Accession number	Coding gene*
	***tuf* sequences**		
Bacteria			
Actinobacillus actinomycetemcomitans	HK1651	Genome project[2]	*tuf*
Actinobacillus actinomycetemcomitans	HK1651	Genome project[2]	*tuf* (EF-G)
Agrobacterium tumefaciens		X99673	*tuf*
Agrobacterium tumefaciens		X99673	*tuf* (EF-G)
Agrobacterium tumefaciens		X99674	*tuf*
Anacystis nidulans	PCC 6301	X17442	*tuf*
Aquifex aeolicus	VF5	AE000669	*tuf*
Aquifex aeolicus	VF5	AE000669	*tuf* (EF-G)
Aquifex pyrophilus		Genome project[2]	*tuf* (EF-G)
Aquifex pyrophilus		Y15787	*tuf*
Bacillus anthracis	Ames	Genome project[2]	*tuf*
Bacillus anthracis	Ames	Genome project[2]	*tuf* (EF-G)
Bacillus halodurans	C-125	AB017508	*tuf*
Bacillus halodurans	C-125	AB017508	*tuf* (EF-G)
Bacillus stearothermophilus	CCM 2184	AJ000260	*tuf*
Bacillus subtilis	168	D64127	*tuf*
Bacillus subtilis	168	D64127	*tuf* (EF-G)
Bacillus subtilis	DSM 10	Z99104	*tuf*
Bacillus subtilis	DSM 10	Z99104	*tuf* (EF-G)
Bacteroides forsythus	ATCC 43037	AB035466	*tuf*
Bacteroides fragilis	DSM 1151	-[1]	*tuf*
Bordetella bronchiseptica	RB50	Genome project[2]	*tuf*
Bordetella pertussis	Tohama 1	Genome project[2]	*tuf*
Bordetella pertussis	Tohama 1	Genome project[2]	*tuf* (EF-G)
Borrelia burdorgferi	B31	U78193	*tuf*
Borrelia burgdorferi		AE001155	*tuf* (EF-G)
Brevibacterium linens	DSM 20425	X76863	*tuf*
Buchnera aphidicola	Ap	Y12307	*tuf*
Burkholderia pseudomallei	K96243	Genome project[2]	*tuf* (EF-G)
Campylobacter jejuni	NCTC 11168	Y17167	*tuf*
Campylobacter jejuni	NCTC 11168	CJ11168X2	*tuf* (EF-G)
Chlamydia pneumoniae	CWL029	AE001592	*tuf*
Chlamydia pneumoniae	CWL029	AE001639	*tuf* (EF-G)
Chlamydia trachomatis		M74221	*tuf*
Chlamydia trachomatis	D/UW-3/CX	AE001317	*tuf* (EF-G)
Chlamydia trachomatis	D/UW-3/CX	AE001305	*tuf*
Chlamydia trachomatis	F/IC-Cal-13	L22216	*tuf*
Chlorobium vibrioforme	DSM 263	X77033	*tuf*
Chloroflexus aurantiacus	DSM 636	X76865	*tuf*
Clostridium acetobutylicum	ATCC 824	Genome project[2]	*tuf*
Clostridium difficile	630	Genome project[2]	*tuf*
Clostridium difficile	630	Genome project[2]	*tuf* (EF-G)
Corynebacterium diphtheriae	NCTC 13129	Genome project[2]	*tuf*
Corynebacterium diphtheriae	NCTC 13129	Genome project[2]	*tuf* (EF-G)
Corynebacterium glutamicum	ASO 19	X77034	*tuf*
Corynebacterium glutamicum	MJ-233	E09634	*tuf*
Coxiella burnetii	Nine Mile phase I	AF136604	*tuf*
Cytophaga lytica	DSM 2039	X77035	*tuf*
Deinococcus radiodurans	R1	AE001891	*tuf* (EF-G)
Deinococcus radiodurans	R1	AE180092	*tuf*

Table 11. Microbial species f r which *tuf* and/ r *atpD* and/or *recA* sequences are available in public databases (continued).

Species	Strain	Accession number	Coding gene*
Deinococcus radiodurans	R1	AE002041	*tuf*
Deinonema sp.		-[1]	*tuf*
Eikenella corrodens	ATCC 23834	Z12610	*tuf*
Eikenella corrodens	ATCC 23834	Z12610	*tuf* (EF-G)
Enterococcus faecalis		Genome project[2]	*tuf* (EF-G)
Escherichia coli		J01690	*tuf*
Escherichia coli		J01717	*tuf*
Escherichia coli		X00415	*tuf* (EF-G)
Escherichia coli		X57091	*tuf*
Escherichia coli	K-12 MG1655	U00006	*tuf*
Escherichia coli	K-12 MG1655	U00096	*tuf*
Escherichia coli	K-12 MG1655	AE000410	*tuf* (EF-G)
Fervidobacterium islandicum	DSM 5733	Y15788	*tuf*
Fibrobacter succinogenes	S85	X76866	*tuf*
Flavobacterium ferrigeneum	DSM 13524	X76867	*tuf*
Flexistipes sinusarabici		X59461	*tuf*
Gloeobacter violaceus	PCC 7421	U09433	*tuf*
Gloeothece sp.	PCC 6501	U09434	*tuf*
Haemophilus actinomycetemcomitans	HK1651	Genome project[2]	*tuf*
Haemophilus ducreyi	35000	AF087414	*tuf* (EF-G)
Haemophilus influenzae	Rd	U32739	*tuf*
Haemophilus influenzae	Rd	U32746	*tuf*
Haemophilus influenzae	Rd	U32739	*tuf* (EF-G)
Helicobacter pylori	26695	AE000511	*tuf*
Helicobacter pylori	J99	AE001539	*tuf* (EF-G)
Helicobacter pylori	J99	AE001541	*tuf*
Herpetosiphon aurantiacus	Hpga1	X76868	*tuf*
Klebsiella pneumoniae	M6H 78578	Genome project[2]	*tuf*
Klebsiella pneumoniae	M6H 78578	Genome project[2]	*tuf* (EF-G)
Lactobacillus paracasei		E13922	*tuf*
Legionella pneumophila	Philadelphia-1	Genome project[2]	*tuf*
Leptospira interrogans		AF115283	*tuf*
Leptospira interrogans		AF115283	*tuf* (EF-G)
Micrococcus luteus	IFO 3333	M17788	*tuf* (EF-G)
Micrococcus luteus	IFO 3333	M17788	*tuf*
Moraxella sp.	TAC II 25	AJ249258	*tuf*
Mycobacterium avium	104	Genome project[2]	*tuf*
Mycobacterium avium	104	Genome project[2]	*tuf* (EF-G)
Mycobacterium bovis	AF2122/97	Genome project[2]	*tuf*
Mycobacterium bovis	AF2122/97	Genome project[2]	*tuf* (EF-G)
Mycobacterium leprae		L13276	*tuf*
Mycobacterium leprae		Z14314	*tuf*
Mycobacterium leprae		Z14314	*tuf* (EF-G)
Mycobacterium leprae	Thai 53	D13869	*tuf*
Mycobacterium tuberculosis	Erdmann	S40925	*tuf*
Mycobacterium tuberculosis	H37Rv	AL021943	*tuf* (EF-G)
Mycobacterium tuberculosis	H37Rv	Z84395	*tuf*
Mycobacterium tuberculosis	y42	AD000005	*tuf*
Mycobacterium tuberculosis	CSU#93	Genome project[2]	*tuf*
Mycobacterium tuberculosis	CSU#93	Genome project[2]	*tuf* (EF-G)
Mycoplasma capricolum	PG-31	X16462	*tuf*
Mycoplasma genitalium	G37	U39732	*tuf*
Mycoplasma genitalium	G37	U39689	*tuf* (EF-G)
Mycoplasma hominis		X57136	*tuf*
Mycoplasma hominis	PG21	M57675	*tuf*

Table 11. Micr bial sp cies for which *tuf* and/or *atpD* and/ r *recA* s quences ar availabl in public databases (continu d).

Species	Strain	Accession number	Coding gene*
Mycoplasma pneumoniae	M129	AE000019	*tuf*
Mycoplasma pneumoniae	M129	AE000058	*tuf* (EF-G)
Neisseria gonorrhoeae	MS11	L36380	*tuf*
Neisseria gonorrhoeae	MS11	L36380	*tuf* (EF-G)
Neisseria meningitidis	Z2491	Genome project[2]	*tuf* (EF-G)
Neisseria meningitidis	Z2491	Genome project[2]	*tuf*
Pasteurella multocida	Pm70	Genome project[2]	*tuf*
Peptococcus niger	DSM 20745	X76869	*tuf*
Phormidium ectocarpi	PCC 7375	U09443	*tuf*
Planobispora rosea	ATCC 53773	U67308	*tuf*
Planobispora rosea	ATCC 53733	X98830	*tuf*
Planobispora rosea	ATCC 53733	X98830	*tuf* (EF-G)
Plectonema boryanum	PCC 73110	U09444	*tuf*
Porphyromonas gingivalis	W83	Genome project[2]	*tuf*
Porphyromonas gingivalis	W83	Genome project[2]	*tuf* (EF-G)
Porphyromonas gingivalis	FDC 381	AB035461	*tuf*
Porphyromonas gingivalis	W83	AB035462	*tuf*
Porphyromonas gingivalis	SUNY 1021	AB035463	*tuf*
Porphyromonas gingivalis	A7A1-28	AB035464	*tuf*
Porphyromonas gingivalis	ATCC 33277	AB035465	*tuf*
Porphyromonas gingivalis	ATCC 33277	AB035471	*tuf* (EF-G)
Prochlorothrix hollandica		U09445	*tuf*
Pseudomonas aeruginosa	PAO-1	Genome project[2]	*tuf*
Pseudomonas putida		Genome project[2]	*tuf*
Rickettsia prowazekii	Madrid E	AJ235272	*tuf*
Rickettsia prowazekii	Madrid E	AJ235270	*tuf* (EF-G)
Rickettsia prowazekii	Madrid E	Z54171	*tuf* (EF-G)
Salmonella choleraesuis subsp. *choleraesuis* serotype *Typhimurium*		X64591	*tuf* (EF-G)
Salmonella choleraesuis subsp. *choleraesuis* serotype *Typhimurium*	LT2 trpE91	X55116	*tuf*
Salmonella choleraesuis subsp. *choleraesuis* serotype *Typhimurium*	LT2 trpE91	X55117	*tuf*
Serpulina hyodysenteriae	B204	U51635	*tuf*
Serratia marcescens		AF058451	*tuf*
Shewanella putrefaciens	DSM 50426	-[1]	*tuf*
Shewanella putrefaciens	MR-1	Genome project[2]	*tuf*
Spirochaeta aurantia	DSM 1902	X76874	*tuf*
Staphylococcus aureus		AJ237696	*tuf* (EF-G)
Staphylococcus aureus	EMRSA-16	Genome project[2]	*tuf*
Staphylococcus aureus	NCTC 8325	Genome project[2]	*tuf*
Staphylococcus aureus	COL	Genome project[2]	*tuf*
Staphylococcus aureus	EMRSA-16	Genome project[2]	*tuf* (EF-G)
Stigmatella aurantiaca	DW4	X82820	*tuf*
Stigmatella aurantiaca	Sg a1	X76870	*tuf*
Streptococcus mutans	GS-5 Kuramitsu	U75481	*tuf*
Streptococcus mutans	UAB159	Genome project[2]	*tuf*
Streptococcus oralis	NTCC 11427	P331701	*tuf*
Streptococcus pyogenes		Genome project[2]	*tuf* (EF-G)
Streptococcus pyogenes	M1-GAS	Genome project[2]	*tuf*
Streptomyces aureofaciens	ATCC 10762	AF007125	*tuf*
Streptomyces cinnamoneus	Tue89	X98831	*tuf*
Streptomyces coelicolor	A3(2)	AL031013	*tuf* (EF-G)
Streptomyces coelicolor	A3(2)	X77039	*tuf* (EF-G)
Streptomyces coelicolor	M145	X77039	*tuf*

Table 11. Micr bial sp ci s f r which *tuf* and/or *atpD* and/ r *recA* s quences are available in public databas s (continued).

Species	Strain	Accession number	Coding gene*
Streptomyces collinus	BSM 40733	S79408	*tuf*
Streptomyces netropsis	Tu1063	AF153618	*tuf*
Streptomyces ramocissimus		X67057	*tuf*
Streptomyces ramocissimus		X67058	*tuf*
Streptomyces ramocissimus		X67057	*tuf* (EF-G)
Synechococcus sp.	PCC 6301	X17442	*tuf* (EF-G)
Synechococcus sp.	PCC 6301	X17442	*tuf*
Synechocystis sp.	PCC 6803	D90913	*tuf* (EF-G)
Synechocystis sp.	PCC 6803	D90913	*tuf*
Synechocystis sp.	PCC 6803	X65159	*tuf* (EF-G)
Taxeobacter occealus	Myx 2105	X77036	*tuf*
Thermotoga maritima		Genome project[2]	*tuf* (EF-G)
Thermotoga maritima		M27479	*tuf*
Thermus aquaticus	EP 00276	X66322	*tuf*
Thermus thermophilus	HB8	X16278	*tuf* (EF-G)
Thermus thermophilus	HB8	X05977	*tuf*
Thermus thermophilus	HB8	X06657	*tuf*
Thiomonas cuprina	DSM 5495	U78300	*tuf*
Thiomonas cuprina	DSM 5495	U78300	*tuf* (EF-G)
Thiomonas cuprina	Hoe5	X76871	*tuf*
Treponema denticola		Genome project[2]	*tuf*
Treponema denticola		Genome project[2]	*tuf* (EF-G)
Treponema pallidum		AE001202	*tuf*
Treponema pallidum		AE001222	*tuf* (EF-G)
Treponema pallidum		AE001248	*tuf* (EF-G)
Ureaplasma urealyticum	ATCC 33697	Z34275	*tuf*
Ureaplasma urealyticum	serovar 3 biovar 1	AE002151	*tuf*
Ureaplasma urealyticum	serovar 3 biovar 1	AE002151	*tuf* (EF-G)
Vibrio cholerae	N16961	Genome project[2]	*tuf*
Wolinella succinogenes	DSM 1740	X76872	*tuf*
Yersinia pestis	CO-92	Genome project[2]	*tuf*
Yersinia pestis	CO-92	Genome project[2]	*tuf* (EF-G)
Archaebacteria			
Archaeoglobus fulgidus		Genome project[2]	*tuf* (EF-G)
Halobacterium marismortui		X16677	*tuf*
Methanobacterium thermoautrophicum	delta H	AE000877	*tuf*
Methanococcus jannaschii	ATCC 43067	U67486	*tuf*
Methanococcus vannielii		X05698	*tuf*
Pyrococcus abyssi	Orsay	AJ248285	*tuf*
Thermoplasma acidophilum	DSM 1728	X53866	*tuf*
Fungi			
Absidia glauca	CBS 101.48	X54730	*tuf* (EF-1)
Arxula adeninivorans	Ls3	Z47379	*tuf* (EF-1)
Aspergillus oryzae	KBN616	AB007770	*tuf* (EF-1)
Aureobasidium pullulans	R106	U19723	*tuf* (EF-1)
Candida albicans	SC5314	Genom project[2]	*tuf* (M)
Candida albicans	SC5314	M29934	*tuf* (EF-1)
Candida albicans	SC5314	M29935	*tuf* (EF-1)
Cryptococcus neoformans	B3501	U81803	*tuf* (EF-1)

Table 11. Micr bial speci s for which *tuf* and/or *atpD* and/or *recA* s qu nces ar availabl in public databas s (c ntinu d).

Species	Strain	Accession number	Coding gene*
Cryptococcus neoformans	M1-106	U81804	*tuf* (EF-1)
Eremothecium gossypii	ATCC 10895	X73978	*tuf* (EF-1)
Eremothecium gossypii		A29820	*tuf* (EF-1)
Fusarium oxysporum	NRRL 26037	AF008498	*tuf* (EF-1)
Histoplasma capsulatum	186AS	U14100	*tuf* (EF-1)
Podospora anserina		X74799	*tuf* (EF-1)
Podospora curvicolla	VLV	X96614	*tuf* (EF-1)
Prototheca wickerhamii	263-11	AJ245645	*tuf* (EF-1)
Puccinia graminis	race 32	X73529	*tuf* (EF-1)
Reclinomonas americana	ATCC 50394	AF007261	*tuf* (M)
Rhizomucor racemosus	ATCC 1216B	X17475	*tuf* (EF-1)
Rhizomucor racemosus	ATCC 1216B	J02605	*tuf* (EF-1)
Rhizomucor racemosus	ATCC 1216B	X17476	*tuf* (EF-1)
Rhodotorula mucilaginosa		AF016239	*tuf* (EF-1)
Saccharomyces cerevisiae		K00428	*tuf* (M)
Saccharomyces cerevisiae		M59369	*tuf* (EF-G)
Saccharomyces cerevisiae		X00779	*tuf* (EF-1)
Saccharomyces cerevisiae		X01638	*tuf* (EF-1)
Saccharomyces cerevisiae		M10992	*tuf* (EF-1)
Saccharomyces cerevisiae	Alpha S288	X78993	*tuf* (EF-1)
Saccharomyces cerevisiae		M15666	*tuf* (EF-1)
Saccharomyces cerevisiae		Z35987	*tuf* (EF-1)
Saccharomyces cerevisiae	S288C (AB972)	U51033	*tuf* (EF-1)
Schizophyllum commune	1-40	X94913	*tuf* (EF-1)
Schizosaccharomyces pombe	972h-	AL021816	*tuf* (EF-1)
Schizosaccharomyces pombe	972h-	AL021813	*tuf* (EF-1)
Schizosaccharomyces pombe	972h-	D82571	*tuf* (EF-1)
Schizosaccharomyces pombe		U42189	*tuf* (EF-1)
Schizosaccharomyces pombe	PR745	D89112	*tuf* (EF-1)
Sordaria macrospora	000	X96615	*tuf* (EF-1)
Trichoderma reesei	QM9414	Z23012	*tuf* (EF-1)
Yarrowia lipolytica		AF054510	*tuf* (EF-1)
Parasites			
Blastocystis hominis	HE87-1	D64080	*tuf* (EF-1)
Cryptosporidium parvum		U69697	*tuf* (EF-1)
Eimeria tenella	LS18	AI755521	*tuf* (EF-1)
Entamoeba histolytica	HM1:IMSS	X83565	*tuf* (EF-1)
Entamoeba histolytica	NIH 200	M92073	*tuf* (EF-1)
Giardia lamblia		D14342	*tuf* (EF-1)
Kentrophoros sp.		AF056101	*tuf* (EF-1)
Leishmania amazonensis	IFLA/BR/67/PH8	M92653	*tuf* (EF-1)
Leishmania braziliensis		U72244	*tuf* (EF-1)
Onchocerca volvulus		M64333	*tuf* (EF-1)
Porphyra purpurea	Avonport	U08844	*tuf* (EF-1)
Plasmodium berghei	ANKA	AJ224150	*tuf* (EF-1)
Plasmodium falciparum	K1	X60488	*tuf* (EF-1)
Plasmodium knowlesi	line H	AJ224153	*tuf* (EF-1)
Toxoplasma gondii	RH	Y11431	*tuf* (EF-1)
Trichomonas tenax	ATCC 30207	D78479	*tuf* (EF-1)
Trypanosoma brucei	LVH/75/ USAMRU-K/18	U10562	*tuf* (EF-1)
Trypanosoma cruzi	Y	L76077	*tuf* (EF-1)

Table 11. Microbial species f r which *tuf* and/ r *atpD* and/ r *recA* sequ nces ar availabl in public databas s (continued).

Species	Strain	Accession number	Coding gene*
Human and plants			
Arabidopsis thaliana	Columbia	X89227	*tuf* (EF-1)
Glycine max	Ceresia	X89058	*tuf* (EF-1)
Glycine max	Ceresia	Y15107	*tuf* (EF-1)
Glycine max	Ceresia	Y15108	*tuf* (EF-1)
Glycine max	Maple Arrow	X66062	*tuf* (EF-1)
Homo sapiens		X03558	*tuf* (EF-1)
Pyramimonas disomata		AB008010	*tuf*
	***atpD* sequences**		
Bacteria			
Acetobacterium woodi	DSM 1030	U10505	*atpD*
Actinobacillus actinomycetemcomitans	HK1651	Genome project[2]	*atpD*
Bacillus anthracis	Ames	Genome project[2]	*atpD*
Bacillus firmus	OF4	M60117	*atpD*
Bacillus megaterium	QM B1551	M20255	*atpD*
Bacillus stearothermophilus		D38058	*atpD*
Bacillus stearothermophilus	IFO1035	D38060	*atpD*
Bacillus subtilis	168	Z28592	*atpD*
Bacteroides fragilis	DSM 2151	M22247	*atpD*
Bordetella bronchiseptica	RB50	Genome project[2]	*atpD*
Bordetella pertussis	Tohama 1	Genome project[2]	*atpD*
Borrelia burgdorferi	B31	AE001122	*atpD* (V)
Burkholderia cepacia	DSM50181	X76877	*atpD*
Burkholderia pseudomallei	K96243	Genome project[2]	*atpD*
Campylobacter jejuni	NCTC 11168	CJ11168X1	*atpD*
Chlamydia pneumoniae		Genome project[2]	*atpD* (V)
Chlamydia trachomatis	MoPn	Genome project[2]	*atpD* (V)
Chlorobium vibrioforme	DSM 263	X76873	*atpD*
Citrobacter freundii	JEO503	AF037156	*atpD*
Clostridium acetobutylicum	ATCC 824	Genome project[2]	*atpD*
Clostridium acetobutylicum	DSM 792	AF101055	*atpD*
Clostridium difficile	630	Genome project[2]	*atpD*
Corynebacterium diphtheriae	NCTC13129	Genome project[2]	*atpD*
Corynebacterium glutamicum	ASO 19	X76875	*atpD*
Corynebacterium glutamicum	MJ-233	E09634	*atpD*
Cytophaga lytica	DSM 2039	M22535	*atpD*
Enterobacter aerogenes	DSM 30053	-[3]	*atpD*
Enterococcus faecalis	V583	Genome project[2]	*atpD* (V)
Enterococcus hirae		M90060	*atpD*
Enterococcus hirae	ATCC 9790	D17462	*atpD* (V)
Escherichia coli		J01594	*atpD*
Escherichia coli		M25464	*atpD*
Escherichia coli		V00267	*atpD*
Escherichia coli		V00311	*atpD*
Escherichia coli	K12 MG1655	L10328	*atpD*
Flavobacterium ferrugineum	DSM 13524	-[3]	*atpD*
Haemophilus actinomycetemcomitans		Genome project[2]	*atpD*
Haemophilus influenzae	Rd	U32730	*atpD*
Helicobacter pylori	NCTC 11638	AF004014	*atpD*

Table 11. Microbial speci s for which *tuf* and/ r *atpD* and/or *recA* sequences ar available in public databases (c ntinu d).

Species	Strain	Accession number	Coding gene*
Helicobacter pylori	26695	Genome project[2]	*atpD*
Helicobacter pylori	J99	Genome project[2]	*atpD*
Klebsiella pneumoniae	M6H 78578	Genome project[2]	*atpD*
Lactobacillus casei	DSM 20021	X64542	*atpD*
Legionella pneumophila	Philadelphia-1	Genome project[2]	*atpD*
Moorella thermoacetica	ATCC 39073	U64318	*atpD*
Mycobacterium avium	104	Genome project[2]	*atpD*
Mycobacterium bovis	AF2122/97	Genome project[2]	*atpD*
Mycobacterium leprae		U15186	*atpD*
Mycobacterium leprae		Genome project[2]	*atpD*
Mycobacterium tuberculosis	H37Rv	Z73419	*atpD*
Mycobacterium tuberculosis	CSU#93	Genome project[2]	*atpD*
Mycoplasma gallisepticum		X64256	*atpD*
Mycoplasma genitalium	G37	U39725	*atpD*
Mycoplasma pneumoniae	M129	U43738	*atpD*
Neisseria gonorrhoeae	FA 1090	Genome project[2]	*atpD*
Neisseria meningitidis	Z2491	Genome project[2]	*atpD*
Pasteurella multocida	Pm70	Genome project[2]	*atpD*
Pectinatus frisingensis	DSM 20465	X64543	*atpD*
Peptococcus niger	DSM 20475	X76878	*atpD*
Pirellula marina	IFAM 1313	X57204	*atpD*
Porphyromonas gingivalis	W83	Genome project[2]	*atpD* (V)
Propionigenium modestum	DSM 2376	X58461	*atpD*
Pseudomonas aeruginosa	PAO1	Genome project[2]	*atpD*
Pseudomonas putida		Genome project[2]	*atpD*
Rhodobacter capsulatus	B100	X99599	*atpD*
Rhodospirillum rubrum		X02499	*atpD*
Rickettsia prowazekii	F-12	AF036246	*atpD*
Rickettsia prowazekii	Madrid	Genome project[2]	*atpD*
Ruminococcus albus	7ATCC	AB006151	*atpD*
Salmonella bongori	JEO4162	AF037155	*atpD*
Salmonella bongori	BR1859	AF037154	*atpD*
Salmonella choleraesuis subsp. *arizonae*	S83769	AF037146	*atpD*
Salmonella choleraesuis subsp. *arizonae*	u24	AF037147	*atpD*
Salmonella choleraesuis subsp. *choleraesuis* serotype Dublin	K228	AF037140	*atpD*
Salmonella choleraesuis subsp. *choleraesuis* serotype Dublin	K771	AF037139	*atpD*
Salmonella choleraesuis subsp. *choleraesuis* serotype Infantis	Div36-86	AF037142	*atpD*
Salmonella choleraesuis subsp. *choleraesuis* serotype Tennessee	Div95-86	AF037143	*atpD*
Salmonella choleraesuis subsp. *choleraesuis* serotype Typhimurium	LT2	AF037141	*atpD*
Salmonella choleraesuis subsp. *diarizonae*	DS210/89	AF037149	*atpD*
Salmonella choleraesuis subsp. *diarizonae*	JEO307	AF037148	*atpD*
Salmonella choleraesuis subsp. *diarizonae*	S109671	AF037150	*atpD*
Salmonella choleraesuis subsp. *houtenae*	S84366	AF037151	*atpD*
Salmonella choleraesuis	S84098	AF037152	*atpD*

Table 11. **Microbial species f r which *tuf* and/or *atpD* and/or *recA* sequenc s ar availabl in public databases (continued).**

Species	Strain	Accession number	Coding gene*
subsp. *houtenae*			
Salmonella choleraesuis subsp. *indica*	BR2047	AF037153	*atpD*
Salmonella choleraesuis subsp. *salamae*	NSC72	AF037144	*atpD*
Salmonella choleraesuis subsp. *salamae*	S114655	AF037145	*atpD*
Shewanella putrefaciens	MR-1	Genome project[2]	*atpD*
Staphylococcus aureus	COL	Genome project[2]	*atpD*
Stigmatella aurantiaca	Sga1	X76879	*atpD*
Streptococcus bovis	JB-1	AB009314	*atpD*
Streptococcus mutans	GS-5	U31170	*atpD*
Streptococcus mutans	UAB159	Genome project[2]	*atpD*
Streptococcus pneumoniae	Type 4	Genome project[2]	*atpD* (V)
Streptococcus pneumoniae	Type 4	Genome project[2]	*atpD*
Streptococcus pyogenes	M1-GAS	Genome project[2]	*atpD* (V)
Streptococcus pyogenes	M1-GAS	Genome project[2]	*atpD*
Streptococcus sanguinis	10904	AF001955	*atpD*
Streptomyces lividans	1326	Z22606	*atpD*
Thermus thermophilus	HB8	D63799	*atpD* (V)
Thiobacillus ferrooxidans	ATCC 33020	M81087	*atpD*
Treponema pallidum	Nichols	AE001228	*atpD* (V)
Vibrio alginolyticus		X16050	*atpD*
Vibrio cholerae	N16961	Genome project[2]	*atpD*
Wolinella succinogenes	DSM 1470	X76880	*atpD*
Yersinia enterocolitica	NCTC 10460	AF037157	*atpD*
Yersinia pestis	CO-92	Genome project[2]	*atpD*
Archaebacteria			
Archaeoglobus fulgidus	DSM 4304	AE001023	*atpD* (V)
Halobacterium salinarum		S56356	*atpD* (V)
Haloferax volcanii	WR 340	X79516	*atpD*
Methanococcus jannaschii	DSM 2661	U67477	*atpD* (V)
Methanosarcina barkeri	DSM 800	J04836	*atpD* (V)
Fungi			
Candida albicans	SC5314	Genome project[2]	*atpD*
Candida tropicalis		M64984	*atpD (V)*
Kluyveromyces lactis	2359/152	U37764	*atpD*
Neurospora crassa		X53720	*atpD*
Saccharomyces cerevisiae		M12082	*atpD*
Saccharomyces cerevisiae	X2180-1A	J05409	*atpD* (V)
Schizosaccharomyces pombe	972 h-	S47814	*atpD* (V)
Schizosaccharomyces pombe	972 h-	M57956	*atpD*
Parasit s			
Giardia lamblia	WB	U18938	*atpD*
Plasmodium falciparum	3D7	L08200	*atpD* (V)
Trypanosoma congolense	IL3000	Z25814	*atpD (V)*

Table 11. Microbial species f r which *tuf* and/or *atpD* and/or *recA* s qu nces ar availabl in public databas s (continu d).

Species	Strain	Accession number	Coding gene*
Human and plants			
Homo sapiens		L09234	*atpD* (V)
Homo sapiens		M27132	*atpD*
	***recA* sequences**		
Bacteria			
Acetobacter aceti	no. 1023	S60630	*recA*
Acetobacter altoacetigenes	MH-24	E05290	*recA*
Acetobacter polyoxogenes	NBI 1028	D13183	*recA*
Acholeplasma laidlawii	8195	M81465	*recA*
Acidiphilium facilis	ATCC 35904	D16538	*recA*
Acidothermus cellulolyticus	ATCC 43068	AJ006705	*recA*
Acinetobacter calcoaceticus	BD413/ADP1	L26100	*recA*
Actinobacillus actinomycetemcomitans	HK1651	Genome project[2]	*recA*
Aeromonas salmonicida	A449	U83688	*recA*
Agrobacterium tumefaciens	C58	L07902	*recA*
Allochromatium vinosum		AJ000677	*recA*
Aquifex aeolicus	VF5	AE000775	*recA*
Aquifex pyrophilus	Kol5a	L23135	*recA*
Azotobacter vinelandii		S96898	*recA*
Bacillus stearothermophilus	10	Genome project[2]	*recA*
Bacillus subtilis	PB1831	U87792	*recA*
Bacillus subtilis	168	Z99112	*recA*
Bacteroides fragilis		M63029	*recA*
Bifidobacterium breve	NCFB 2258	AF094756	*recA*
Blastochloris viridis	DSM 133	AF022175	*recA*
Bordetella pertussis	165	X53457	*recA*
Bordetella pertussis	Tohama I	Genome project[2]	*recA*
Borrelia burgdorferi	Sh-2-82	U23457	*recA*
Borrelia burgdorferi	B31	AE001124	*recA*
Brevibacterium flavum	MJ-233	E10390	*recA*
Brucella abortus	2308	L00679	*recA*
Burkholderia cepacia	ATCC 17616	U70431	*recA*
Burkholderia cepacia		D90120	*recA*
Burkholderia pseudomallei	K96243	Genome project[2]	*recA*
Campylobacter fetus subsp. *fetus*	23D	AF020677	*recA*
Campylobacter jejuni	81-176	U03121	*recA*
Campylobacter jejuni	NCTC 11168	AL139079	*recA*
Chlamydia trachomatis	L2	U16739	*recA*
Chlamydia trachomatis	D/UW-3/CX	AE001335	*recA*
Chlamydophila pneumoniae	CWL029	AE001658	*recA*
Chloroflexus aurantiacus	J-10-fl	AF037259	*recA*
Clostridium acetobutylicum		M94057	*recA*
Clostridium perfringens	13	U61497	*recA*
Corynebacterium diphtheriae	NCTC13129	Genome project[2]	*recA*
Corynebacterium glutamicum	AS019	U14965	*recA*
Corynebacterium pseudotuberculosis	C231	U30387	*recA*
Deinococcus radiodurans	KD8301	AB005471	*recA*
Deinococcus radiodurans	R1	U01876	*recA*

Table 11. Microbial species for which *tuf* and/or *atpD* and/ r *recA* s qu nces are availabl in public databases (c ntinued).

Species	Strain	Accession number	Coding gene*
Enterobacter agglomerans	339	L03291	*recA*
Enterococcus faecalis	OGIX	M81466	*recA*
Erwinia carotovora		X55554	*recA*
Escherichia coli		J01672	*recA*
Escherichia coli		X55552	*recA*
Escherichia coli	K-12	AE000354	*recA*
Frankia alni	Arl3	AJ006707	*recA*
Gluconobacter oxydans		U21001	*recA*
Haemophilus influenzae	Rd	U32687	*recA*
Haemophilus influenzae	Rd	U32741	*recA*
Haemophilus influenzae	Rd	L07529	*recA*
Helicobacter pylori	69A	Z35478	*recA*
Helicobacter pylori	26695	AE000536	*recA*
Helicobacter pylori	J99	AE001453	*recA*
Klebsiella pneumoniae	M6H 78578	Genome project[2]	*recA*
Lactococcus lactis	ML3	M88106	*recA*
Legionella pneumophila		X55453	*recA*
Leptospira biflexa	serovar patoc	U32625	*recA*
Leptospira interrogans	serovar pomona	U29169	*recA*
Magnetospirillum magnetotacticum	MS-1	X17371	*recA*
Methylobacillus flagellatus	MFK1	M35325	*recA*
Methylomonas clara	ATCC 31226	X59514	*recA*
Mycobacterium avium	104	Genome project[2]	*recA*
Mycobacterium bovis	AF122/97	Genome project[2]	*recA*
Mycobacterium leprae		X73822	*recA*
Mycobacterium tuberculosis	H37Rv	X58485	*recA*
Mycobacterium tuberculosis	CSU#93	Genome project[2]	*recA*
Mycoplasma genitalium	G37	U39717	*recA*
Mycoplasma mycoides	GM9	L22073	*recA*
Mycoplasma pneumoniae	ATCC 29342	MPAE000033	*recA*
Mycoplasma pulmonis	KD735	L22074	*recA*
Myxococcus xanthus		L40368	*recA*
Myxococcus xanthus		L40367	*recA*
Neisseria animalis	NCTC 10212	U57910	*recA*
Neisseria cinerea	LCDC 81-176	AJ223869	*recA*
Neisseria cinerea	LNP 1646	U57906	*recA*
Neisseria cinerea	NCTC 10294	AJ223871	*recA*
Neisseria cinerea	Vedros M601	AJ223870	*recA*
Neisseria elongata	CCUG 2131	AJ223882	*recA*
Neisseria elongata	CCUG 4165A	AJ223880	*recA*
Neisseria elongata	NCTC 10660	AJ223881	*recA*
Neisseria elongata	NCTC 11050	AJ223878	*recA*
Neisseria elongata	NHITCC 2376	AJ223877	*recA*
Neisseria elongata subsp. *intermedia*	CCUG 4557	AJ223879	*recA*
Neisseria flava	Bangor 9	AJ223873	*recA*
Neisseria flavescens	LNP 444	U57907	*recA*
Neisseria gonorrhoeae	CH95	U57902	*recA*
Neisseria gonorrhoeae	FA19	X64842	*recA*
Neisseria gonorrhoeae	MS11	X17374	*recA*
Neisseria gonorrhoeae		Genome project[2]	*recA*
Neisseria lactamica	CCUC 7757	AJ223866	*recA*
Neisseria lactamica	CCUG 7852	Y11819	*recA*
Neisseria lactamica	LCDC 77-143	Y11818	*recA*
Neisseria lactamica	LCDC 80-111	AJ223864	*recA*

Table 11. Micr bial species for which *tuf* and/or *atpD* and/or *recA* sequ nces ar availabl in public databases (continued).

Species	Strain	Accession number	Coding gene*
Neisseria lactamica	LCDC 845	AJ223865	*recA*
Neisseria lactamica	NCTC 10617	U57905	*recA*
Neisseria lactamica	NCTC 10618	AJ223863	*recA*
Neisseria meningitidis	44/46	X64849	*recA*
Neisseria meningitidis	Bangor 13	AJ223868	*recA*
Neisseria meningitidis	HF116	X64848	*recA*
Neisseria meningitidis	HF130	X64844	*recA*
Neisseria meningitidis	HF46	X64847	*recA*
Neisseria meningitidis	M470	X64850	*recA*
Neisseria meningitidis	N94II	X64846	*recA*
Neisseria meningitidis	NCTC 8249	AJ223867	*recA*
Neisseria meningitidis	P63	X64845	*recA*
Neisseria meningitidis	S3446	U57903	*recA*
Neisseria meningitidis	FAM18	Genome project[2]	*recA*
Neisseria mucosa	LNP 405	U57908	*recA*
Neisseria mucosa	Vedros M1801	AJ223875	*recA*
Neisseria perflava	CCUG 17915	AJ223876	*recA*
Neisseria perflava	LCDC 85402	AJ223862	*recA*
Neisseria pharyngis var. *flava*	NCTC 4590	U57909	*recA*
Neisseria polysaccharea	CCUG 18031	Y11815	*recA*
Neisseria polysaccharea	CCUG 24845	Y11816	*recA*
Neisseria polysaccharea	CCUG 24846	Y11814	*recA*
Neisseria polysaccharea	INS MA 3008	Y11817	*recA*
Neisseria polysaccharea	NCTC 11858	U57904	*recA*
Neisseria sicca	NRL 30016	AJ223872	*recA*
Neisseria subflava	NRL 30017	AJ223874	*recA*
Paracoccus denitrificans	DSM 413	U59631	*recA*
Pasteurella multocida		X99324	*recA*
Porphyromonas gingivalis	W83	U70054	*recA*
Prevotella ruminicola	JCM 8958	U61227	*recA*
Proteus mirabilis	pG1300	X14870	*recA*
Proteus vulgaris		X55555	*recA*
Pseudomonas aeruginosa		X05691	*recA*
Pseudomonas aeruginosa	PAM 7	X52261	*recA*
Pseudomonas aeruginosa	PAO12	D13090	*recA*
Pseudomonas fluorescens	OE 28.3	M96558	*recA*
Pseudomonas putida		L12684	*recA*
Pseudomonas putida	PpS145	U70864	*recA*
Rhizobium leguminosarum biovar *viciae*	VF39	X59956	*recA*
Rhizobium phaseoli	CNPAF512	X62479	*recA*
Rhodobacter capsulatus	J50	X82183	*recA*
Rhodobacter sphaeroides	2.4.1	X72705	*recA*
Rhodopseudomonas palustris	N 7	D84467	*recA*
Rickettsia prowazekii	Madrid E	AJ235273	*recA*
Rickettsia prowazekii	Madrid E	U01959	*recA*
Serratia marcescens		M22935	*recA*
Shigella flexneri		X55553	*recA*
Shigella sonnei	KNIH104S	AF101227	*recA*
Sinorhizobium meliloti	2011	X59957	*recA*
Staphylococcus aureus		L25893	*recA*
Streptococcus gordonii	Challis V288	L20574	*recA*
Streptococcus mutans	UA96	M81468	*recA*
Streptococcus mutans	GS-5	M61897	*recA*
Streptococcus pneumoniae		Z17307	*recA*

Table 11. Microbial sp cies f r which *tuf* and/or *atpD* and/or *recA* s quences are availabl in public databases (c ntinu d).

Species	Strain	Acc ssion number	Coding gene*
Streptococcus pneumoniae	R800	Z34303	*recA*
Streptococcus pyogenes	NZ131	U21934	*recA*
Streptococcus pyogenes	D471	M81469	*recA*
Streptococcus salivarius subsp. *thermophilus*		M94062	*recA*
Streptomyces ambofaciens	DSM 40697	Z30324	*recA*
Streptomyces coelicolor	A3(2)	AL020958	*recA*
Streptomyces lividans	TK24	X76076	*recA*
Streptomyces rimosus	R6	X94233	*recA*
Streptomyces venezuelae	ATCC10712	U04837	*recA*
Synechococcus sp.	PR6	M29495	*recA*
Synechocystis sp.	PCC6803	D90917	*recA*
Thermotoga maritima		L23425	*recA*
Thermotoga maritima		AE001823	*recA*
Thermus aquaticus		L20095	*recA*
Thermus thermophilus	HB8	D17392	*recA*
Thiobacillus ferrooxidans		M26933	*recA*
Treponema denticola		Genome project[2]	*recA*
Treponema pallidum	Nichols	AE001243	*recA*
Vibrio anguillarum		M80525	*recA*
Vibrio cholerae	017	X71969	*recA*
Vibrio cholerae	2740-80	U10162	*recA*
Vibrio cholerae	569B	L42384	*recA*
Vibrio cholerae	M549	AF117881	*recA*
Vibrio cholerae	M553	AF117882	*recA*
Vibrio cholerae	M645	AF117883	*recA*
Vibrio cholerae	M793	AF117878	*recA*
Vibrio cholerae	M794	AF117880	*recA*
Vibrio cholerae	M967	AF117879	*recA*
Xanthomonas citri	XW47	AF006590	*recA*
Xanthomonas oryzae		AF013600	*recA*
Xenorhabdus bovienii	T228/1	U87924	*recA*
Xenorhabdus nematophilus	AN6	AF127333	*recA*
Yersinia pestis	231	X75336	*recA*
Yersinia pestis	CO-92	Genome project[2]	*recA*
Fungi, parasites, human and plants			
Anabaena variabilis	ATCC 29413	M29680	*recA*
Arabidopsis thaliana		U43652	*recA* (Rad51)
Candida albicans		U39808	*recA* (Dmc1)
Coprinus cinereus	Okayama-7	U21905	*recA* (Rad51)
Emericella nidulans		Z80341	*recA* (Rad51)
Gallus gallus		L09655	*recA* (Rad51)
Homo sapiens		D13804	*recA* (Rad51)
Homo sapiens		D63882	*recA* (Dmc1)
Leishmania major	Friedlin	AF062379	*recA* (Rad51)
Leishmania major	Friedlin	AF062380	*recA* (Dmc1)
Mus musculus		D58419	*recA* (Dmc1)
Neurospora crassa	74-OR23-1A	D29638	*recA* (Rad51)
Saccharomyces cerevisiae		D10023	*recA* (Rad51)
Schizosaccharomyces pombe		Z22691	*recA* (Rad51)
Schizosaccharomyces pombe	972h-	AL021817	*recA* (Dmc1)
Tetrahymena thermophila	PB9R	AF064516	*recA* (Rad51)

Tabl 11. Microbial species f r which *tuf* and/ r *atpD* and/or *recA* sequenc s are available in public databases (continued).

Species	Strain	Accession number	Coding gene*
Trypanosoma brucei	stock 427	Y13144	*recA* (Rad51)
Ustilago maydis		U62484	*recA* (Rad51)
Xenopus laevis		D38488	*recA* (Rad51)
Xenopus laevis		D38489	*recA* (Rad51)

* *tuf* indicates *tuf* sequences, including *tuf* genes, *fusA* genes and *fusA-tuf* intergenic spacers.
tuf (C) indicates *tuf* sequences divergent from main (usually A and B) copies of the elongation factor-Tu
tuf (EF-1) indicates *tuf* sequences of the eukaryotic type (elongation factor 1α)
tuf (M) indicates *tuf* sequences from organellar (mostly mitochondrial) origin
atpD indicates *atpD* sequences ot the F-type
atpD (V) indicates *atpD* sequences of the V-Type
recA indicates *recA* sequences
recA (Rad51) indicates *rad51* sequences or homologs
recA (Dmc1) indicates *dmc1* sequences or homologs
[1] Nucleotides sequences published in Arch. Microbiol. 1990 **153**:241-247
[2] These sequences are from theTIGR database (http://www.tigr.org/tdb/tdb.html)
[3] Nucleotides sequences published in FEMS Microbiology Letters 1988 **50**:101-106

Table 12. Bacterial species used to t st th specificity of the *Staphylococcus*-specific amplification prim rs deriv d fr m *tuf* s quenc s.

Strain	Reference number	Strain	Reference number
Staphylococcal species (n=27)		**Other Gram-positive bacteria (n=20)**	
Staphylococcus arlettae	ATCC 43957	*Bacillus subtilis*	ATCC 27370
Staphylococcus aureus subsp. *anaerobius*	ATCC 35844	*Enterococcus avium*	ATCC 14025
Staphylococcus aureus subsp. *aureus*	ATCC 43300	*Enterococcus durans*	ATCC 19432
Staphylococcus auricularis	ATCC 33753	*Enterococcus faecalis*	ATCC 19433
Staphylococcus capitis subsp. *capitis*	ATCC 27840	*Enterococcus faecium*	ATCC 19434
Staphylococcus caprae	ATCC 35538	*Enterococcus flavescens*	ATCC 49996
Staphylococcus carnosus	ATCC 51365	*Enterococcus gallinarum*	ATCC 49573
Staphylococcus chromogenes	ATCC 43764	*Lactobacillus acidophilus*	ATCC 4356
Staphylococcus cohnii subsp. *urealyticum*	DSM 20260	*Lactococcus lactis*	ATCC 11454
Staphylococcus delphini	ATCC 49171	*Listeria innocua*	ATCC 33090
Staphylococcus epidermidis	ATCC 14990	*Listeria ivanovii*	ATCC 19119
Staphylococcus equorum	ATCC 43958	*Listeria monocytogenes*	ATCC 15313
Staphylococcus felis	ATCC 49168	*Macrococcus caseolyticus*	ATCC 13548
Staphylococcus gallinarum	ATCC 35539	*Streptococcus agalactiae*	ATCC 13813
Staphylococcus haemolyticus	ATCC 29970	*Streptococcus anginosus*	ATCC 33397
Staphylococcus hominis	ATCC 27844	*Streptococcus bovis*	ATCC 33317
Staphylococcus hyicus	ATCC 11249	*Streptococcus mutans*	ATCC 25175
Staphylococcus intermedius	ATCC 29663	*Streptococcus pneumoniae*	ATCC 6303
Staphylococcus kloosis	ATCC 43959	*Streptococcus pyogenes*	ATCC 19615
Staphylococcus lentus	ATCC 29070	*Streptococcus salivarius*	ATCC 7073
Staphylococcus lugdunensis	ATCC 43809		
Staphylococcus saprophyticus	ATCC 15305		
Staphylococcus schleiferi subsp. *coagulans*	ATCC 49545		
Staphylococcus sciuri subsp. *sciuri*	ATCC 29060		
Staphylococcus simulans	ATCC 27848		
Staphylococcus warneri	ATCC 27836		
Staphylococcus xylosus	ATCC 29971		
Gram-negative bacteria (n=33)			
Acinetobacter baumannii	ATCC 19606	*Morganella morganii*	ATCC 25830
Bacteroides distasonis	ATCC 8503	*Neisseria gonorrhoeae*	ATCC 35201
Bacteroides fragilis	ATCC 25285	*Neisseria meningitidis*	ATCC 13077
Bulkholderia cepacia	ATCC 25416	*Proteus mirabilis*	ATCC 25933
Bordetella pertussis	ATCC 9797	*Proteus vulgaris*	ATCC 13315
Citrobacter freundii	ATCC 8090	*Providencia rettgeri*	ATCC 9250
Enterobacter aerogenes	ATCC 13048	*Providencia stuartii*	ATCC 29914
Enterobacter cloacae	ATCC 13047	*Pseudomonas aeruginosa*	ATCC 27853
Escherichia coli	ATCC 25922	*Pseudomonas fluorencens*	ATCC 13525
Haemophilus influenzae	ATCC 8907	*Salmonella choleraesuis*	ATCC 7001
Haemophilus parahaemolyticus	ATCC 10014	*Salmonella typhimurium*	ATCC 14028
Haemophilus parainfluenzae	ATCC 7901	*Serratia marcescens*	ATCC 8100
Hafnia alvei	ATCC 13337	*Shigella flexneri*	ATCC 12022
Kingella indologenes	ATCC 25869	*Shigella sonnei*	ATCC 29930
Klebsiella oxytoca	ATCC 13182	*Stenotrophomonas maltophilia*	ATCC 13843
Klebsiella pneumoniae	ATCC 13883	*Yersinia enterocolitica*	ATCC 9610
Moraxella catarrhalis	ATCC 25240		

Table 13. Bact rial sp cies us d to test the sp cificity of the penicillin-r sistant *Streptococcus pneumoniae* assay.

Strain	Reference number	Strain	Reference number
Gram-positive species (n=67)			
Abiotrophia adiacens	ATCC 49175	*Staphylococcus hominis*	ATCC 27844
Abiotrophia defectiva	ATCC 49176	*Staphylococcus lugdunensis*	ATCC 43809
Actinomyces pyogenes	ATCC 19411	*Staphylococcus saprophyticus*	ATCC 15305
Bacillus anthracis	ATCC 4229	*Staphylococcus simulans*	ATCC 27848
Bacillus cereus	ATCC 14579	*Staphylococcus. warneri*	ATCC 27836
Bifidobacterium breve	ATCC 15700	*Streptococcus acidominimus*	ATCC 51726
Clostridium difficile	ATCC 9689	*Streptococcus agalactiae*	ATCC 12403
Enterococcus avium	ATCC 14025	*Streptococcus anginosus*	ATCC 33397
Enterococcus casseliflavus	ATCC 25788	*Streptococcus bovis*	ATCC 33317
Enterococcus dispar	ATCC 51266	*Streptococcus constellatus*	ATCC 27823
Enterococcus durans	ATCC 19432	*Streptococcus cricetus*	ATCC 19624
Enterococcus faecalis	ATCC 29212	*Streptococcus cristatus*	ATCC 51100
Enterococcus faecium	ATCC 19434	*Streptococcus downei*	ATCC 33748
Enterococcus flavescens	ATCC 49996	*Streptococcus dysgalactiae*	ATCC 43078
Enterococcus gallinarum	ATCC 49573	*Streptococcus equi*	ATCC 9528
Enterococcus hirae	ATCC 8043	*Streptococcus ferus*	ATCC 33477
Enterococcus mundtii	ATCC 43186	*Streptococcus gordonii*	ATCC 10558
Enterococcus raffinosus	ATCC 49427	*Streptococcus intermedius*	ATCC 27335
Lactobacillus lactis	ATCC 19435	*Streptococcus mitis*	ATCC 903
Lactobacillus monocytogenes	ATCC 15313	*Streptococcus mitis*	LSPQ 2583
Mobiluncus curtisii	ATCC 35242	*Streptococcus mitis*	ATCC 49456
Peptococcus niger	ATCC 27731	*Streptococcus mutans*	ATCC 27175
Peptostreptococcus acones	ATCC 6919	*Streptococcus oralis*	ATCC 10557
Peptostreptococcus anaerobius	ATCC 27337	*Streptococcus oralis*	ATCC 9811
Peptostreptococcus asaccharolyticus	ATCC 2639	*Streptococcus oralis*	ATCC 35037
		Streptococcus parasanguinis	ATCC 15912
Peptostreptococcus lactolyticus	ATCC 51172	*Streptococcus parauberis*	ATCC 6631
Peptostreptococcus magnus	ATCC 15794	*Streptococcus rattus*	ATCC 15912
Peptostreptococcus prevotii	ATCC 9321	*Streptococcus salivarius*	ATCC 7073
Peptostreptococcus tetradius	ATCC 35098	*Streptococcus sanguinis*	ATCC10556
Staphylococcus aureus	ATCC 25923	*Streptococcus suis*	ATCC 43765
Staphylococcus capitis	ATCC 27840	*Streptococcus uberis*	ATCC 19436
Staphylococcus epidermidis	ATCC 14990	*Streptococcus vestibularis*	ATCC 49124
Staphylococcus haemolyticus	ATCC 29970		
Gram-negative species (n=33)			
Actinetobacter baumannii	ATCC 19606	*Moraxella morganii*	ATCC 13077
Bordetella pertussis	ATCC 9797	*Neisseria gonorrhoeae*	ATCC 35201
Citrobacter diversus	ATCC 27028	*Neisseria meningitidis*	ATCC 13077
Citrobacter freundii	ATCC 8090	*Proteus mirabilis*	ATCC 25933
Enterobacter aerogenes	ATCC 13048	*Proteus vulgaris*	ATCC 13315
Enterobacter agglomerans	ATCC 27155	*Providencia alcalifaciens*	ATCC 9886
Enterobacter cloacae	ATCC 13047	*Providencia rettgeri*	ATCC 9250
Escherichia coli	ATCC 25922	*Providencia rustigianii*	ATCC 33673
Haemophilus ducreyi	ATCC 33940	*Providencia stuartii*	ATCC 33672
Haemophilus haemolyticus	ATCC 33390	*Pseudomonas aeruginosa*	ATCC 35554
Haemophilus influenzae	ATCC 9007	*Pseudomonas fluorescens*	ATCC 13525
Haemophilus parainfluenzae	ATCC 7901	*Pseudomonas stutzeri*	ATCC 17588
Hafnia alvei	ATCC 13337	*Salmonella typhimurium*	ATCC 14028
Klebsiella oxytoca	ATCC 13182	*Serratia marcescens*	ATCC 13880
Klebsiella pneumoniae	ATCC 13883	*Shigella flexneri*	ATCC 12022
Moraxella atlantae	ATCC 29525	*Yersina enterocolitica*	ATCC 9610
Moraxella catarrhalis	ATCC 43628		

Table 14. Bacterial sp cies (n=104) det ct d by th plat let contaminants assay. Bold characters indicate the major bacterial contaminants found in platelet concentrates.

Abiotrophia adiacens
Abiotrophia defectiva
Acinetobacter baumannii
Acinetobacter lwoffi
Aerococcus viridans
Bacillus anthracis
Bacillus cereus
Bacillus subtilis
Brucella abortus
Burkholderia cepacia
Citrobacter diversus
Citrobacter freundii
Enterobacter aerogenes
Enterobacter agglomerans
Enterobacter cloacae
Enterococcus avium
Enterococcus casseliflavus
Enterococcus dispar
Enterococcus durans
Enterococcus faecalis
Enterococcus faecium
Enterococcus flavescens
Enterococcus gallinarum
Enterococcus mundtii
Enterococcus raffinosus
Enterococcus solitarius
Escherichia coli
Gemella morbillorum
Haemophilus ducreyi
Haemophilus haemolyticus
Haemophilus influenzae
Haemophilus parahaemolyticus
Haemophilus parainfluenzae
Hafnia alvei
Kingella kingae
Klebsiella oxytoca
Klebsiella pneumoniae
Legionella pneumophila
Megamonas hypermegale
Moraxella atlantae
Moraxella catarrhalis
Morganella morganii
Neisseria gonorrheae
Neisseria meningitidis
Pasteurella aerogenes
Pasteurella multocida
Peptostreptococcus magnus
Proteus mirabilis
Providencia alcalifaciens
Providencia rettgeri
Providencia rustigianii
Providencia stuartii
Pseudomonas aeruginosa
Pseudomonas fluorescens
Pseudomonas stutzeri
Salmonella bongori
Salmonella choleraesuis
Salmonella enteritidis
Salmonella gallinarum
Salmonella typhimurium
Serratia liquefaciens
Serratia marcescens
Shigella flexneri
Shigella sonnei
Staphylococcus aureus
Staphylococcus capitis
Staphylococcus epidermidis
Staphylococcus haemolyticus
Staphylococcus hominis
Staphylococcus lugdunensis
Staphylococcus saprophyticus
Staphylococcus simulans
Staphylococcus warneri
Stenotrophomonas maltophilia
Streptococcus acidominimus
Streptococcus agalactiae
Streptococcus anginosus
Streptococcus bovis
Streptococcus constellatus
Streptococcus cricetus
Streptococcus cristatus
Streptococcus dysgalactiae
Streptococcus equi
Streptococcus ferus
Streptococcus gordonii
Streptococcus intermedius
Streptococcus macacae
Streptococcus mitis
Streptococcus mutans
Streptococcus oralis
Streptococcus parasanguinis
Streptococcus parauberis
Streptococcus pneumoniae
Streptococcus pyogenes
Streptococcus ratti
Streptococcus salivarius
Streptococcus sanguinis
Streptococcus sobrinus
Streptococcus uberis
Streptococcus vestibularis
Vibrio cholerae
Yersinia enterocolitica
Yersinia pestis
Yersinia pseudotuberculosis

Table 15. Microorganisms identified by commercial systems[1].

Abiotrophia adiacens (*Streptococcus adjacens*)
Abiotrophia defectiva (*Streptococcus defectivus*)
Achromobacter species
Acidaminococcus fermentans
Acinetobacter alcaligenes
Acinetobacter anitratus
Acinetobacter baumannii
Acinetobacter calcoaceticus
Acinetobacter calcoaceticus biovar *anitratus*
Acinetobacter calcoaceticus biovar *lwoffi*
Acinetobacter genomospecies
Acinetobacter haemolyticus
Acinetobacter johnsonii
Acinetobacter junii
Acinetobacter lwoffii
Acinetobacter radioresistens
Acinetobacter species
Actinobacillus actinomycetemcomitans
Actinobacillus capsulatus
Actinobacillus equuli
Actinobacillus hominis
Actinobacillus lignieresii
Actinobacillus pleuropneumoniae
Actinobacillus species
Actinobacillus suis
Actinobacillus ureae
Actinomyces bovis
Actinomyces israelii
Actinomyces meyeri
Actinomyces naeslundii
Actinomyces neuii subsp. *anitratus*
Actinomyces neuii subsp. *neuii*
Actinomyces odontolyticus
Actinomyces pyogenes
Actinomyces radingae
Actinomyces species
Actinomyces turicensis
Actinomyces viscosus
Aerococcus species
Aerococcus viridans
Aeromonas caviae
Aeromonas hydrophila
Aeromonas hydrophila group
Aeromonas jandaei
Aeromonas salmonicida
Aeromonas salmonicida subsp. *achromogenes*
Aeromonas salmonicida subsp. *masoucida*
Aeromonas salmonicida subsp. *salmonicida*
Aeromonas schubertii
Aeromonas sobria
Aeromonas species
Aeromonas trota
Aeromonas veronii
Aeromonas veronii biovar *sobria*
Aeromonas veronii biovar *veronii*
Agrobacterium radiobacter
Agrobacterium species
Agrobacterium tumefaciens
Alcaligenes denitrificans
Alcaligenes faecalis
Alcaligenes odorans
Alcaligenes odorans (*Alcaligenes faecalis*)
Alcaligenes species
Alcaligenes xylosoxidans
Alcaligenes xylosoxidans subsp. *denitrificans*
Alcaligenes xylosoxidans subsp. *xylosoxidans*
Alloiococcus otitis
Anaerobiospirillum succiniciproducens
Anaerovibrio lipolytica
Arachnia propionica
Arcanobacterium (*Actinomyces*) *bernardiae*
Arcanobacterium (*Actinomyces*) *pyogenes*
Arcanobacterium haemolyticum
Arcobacter cryaerophilus (*Campylobacter cryaerophila*)
Arthrobacter globiformis
Arthrobacter species
Arxiozyma telluris (*Torulopsis pintolopesii*)
Atopobium minutum (*Lactobacillus minutus*)
Aureobacterium species
Bacillus amyloliquefaciens
Bacillus anthracis
Bacillus badius
Bacillus cereus
Bacillus circulans
Bacillus coagulans
Bacillus firmus
Bacillus lentus
Bacillus licheniformis
Bacillus megaterium
Bacillus mycoides
Bacillus pantothenticus
Bacillus pumilus
Bacillus species
Bacillus sphaericus
Bacillus stearothermophilus
Bacillus subtilis
Bacillus thuringiensis
Bacteroides caccae
Bacteroides capillosus
Bacteroides distasonis
Bacteroides eggerthii
Bacteroides fragilis
Bacteroides merdae
Bacteroides ovatus
Bacteroides species
Bacteroides splanchnicus
Bacteroides stercoris
Bacteroides thetaiotaomicron
Bacteroides uniformis
Bacteroides ureolyticus (*B. corrodens*)
Bacteroides vulgatus
Bergeyella (*Weeksella*) *zoohelcum*
Bifidobacterium adolescentis
Bifidobacterium bifidum
Bifidobacterium breve
Bifidobacterium dentium
Bifidobacterium infantis
Bifidobacterium species
Blastoschizomyces (*Dipodascus*) *capitatus*
Bordetella avium
Bordetella bronchiseptica
Bordetella parapertussis
Bordetella pertussis
Bordetella species
Borrelia species
Branhamella (*Moraxella*) *catarrhalis*
Branhamella species
Brevibacillus brevis
Brevibacillus laterosporus
Brevibacterium casei
Brevibacterium epidermidis
Brevibacterium linens
Brevibacterium species
Brevundimonas (*Pseudomonas*) *diminuta*
Brevundimonas (*Pseudomonas*) *vesicularis*
Brevundimonas species
Brochothrix thermosphacta
Brucella abortus
Brucella canis
Brucella melitensis
Brucella ovis
Brucella species
Brucella suis
Budvicia aquatica
Burkholderia (*Pseudomonas*) *cepacia*
Burkholderia (*Pseudomonas*) *gladioli*
Burkholderia (*Pseudomonas*) *mallei*
Burkholderia (*Pseudomonas*) *pseudomallei*
Burkholderia species
Buttiauxella agrestis
Campylobacter coli
Campylobacter concisus
Campylobacter fetus
Campylobacter fetus subsp. *fetus*
Campylobacter fetus subsp. *venerealis*
Campylobacter hyointestinalis
Campylobacter jejuni subsp. *doylei*
Campylobacter jejuni subsp. *jejuni*
Campylobacter lari
Campylobacter lari subsp. UPTC
Campylobacter mucosalis
Campylobacter species
Campylobacter sputorum
Campylobacter sputorum subsp. *bubulus*
Campylobacter sputorum subsp. *fecalis*
Campylobacter sputorum subsp. *sputorum*
Campylobacter upsaliensis
Candida (*Clavispora*) *lusitaniae*
Candida (*Pichia*) *guilliermondii*
Candida (*Torulopsis*) *glabrata*
Candida albicans
Candida boidinii
Candida catenulata
Candida ciferrii
Candida colliculosa
Candida conglobata
Candida curvata (*Cryptococcus curvatus*)
Candida dattila
Candida dubliniensis
Candida famata
Candida globosa
Candida hellenica
Candida holmii
Candida humicola
Candida inconspicua
Candida intermedia
Candida kefyr
Candida krusei
Candida lambica
Candida magnoliae
Candida maris
Candida melibiosica
Candida membranaefaciens
Candida norvegensis
Candida norvegica
Candida parapsilosis
Candida paratropicalis
Candida pellicutosa

Table 15. Microorganism :ntified by commercial systems (continued)

Candida pseudotropicalis
Candida pulcherrima
Candida ravautii
Candida rugosa
Candida sake
Candida silvicola
Candida species
Candida sphaerica
Candida stellatoidea
Candida tenuis
Candida tropicalis
Candida utilis
Candida valida
Candida vini
Candida viswanathii
Candida zeylanoides
Capnocytophaga gingivalis
Capnocytophaga ochracea
Capnocytophaga species
Capnocytophaga sputigena
Cardiobacterium hominis
Carnobacterium divergens
Carnobacterium piscicola
CDC group ED-2
CDC group EF4 (Pasteurella sp.)
CDC group EF-4A
CDC group EF-4B
CDC group EO-Z
CDC group HB-5
CDC group II K-2
CDC group IV C-2 (Bordetella-like)
CDC group M5
CDC group M6
Cedecea davisae
Cedecea lapagei
Cedecea neteri
Cedecea species
Cellulomonas (Oerskovia) turbata
Cellulomonas species
Chlamydia species
Chromobacterium violaceum
Chryseobacterium (Flavobacterium) indologenes
Chryseobacterium (Flavobacterium) meningosepticum
Chryseobacterium gleum
Chryseobacterium species
Chryseomonas indologenes
Citeromyces matritensis
Citrobacter amalonaticus
Citrobacter braakii
Citrobacter diversus
Citrobacter farmeri
Citrobacter freundii
Citrobacter freundii complex
Citrobacter koseri
Citrobacter sedlakii
Citrobacter species
Citrobacter werkmanii
Citrobacter youngae
Clostridium acetobutylicum
Clostridium barati
Clostridium beijerinckii
Clostridium bifermentans
Clostridium botulinum
Clostridium botulinum (NP) B&F
Clostridium botulinum (NP) E
Clostridium botulinum (P) A&H
Clostridium botulinum (P) F
Clostridium botulinum G1
Clostridium botulinum G2
Clostridium butyricum
Clostridium cadaveris
Clostridium chauvoei
Clostridium clostridiiforme
Clostridium difficile
Clostridium fallax
Clostridium glycolicum
Clostridium hastiforme
Clostridium histolyticum
Clostridium innocuum
Clostridium limosum
Clostridium novyi
Clostridium novyi A
Clostridium paraputrificum
Clostridium perfringens
Clostridium putrificum
Clostridium ramosum
Clostridium septicum
Clostridium sordellii
Clostridium species
Clostridium sphenoides
Clostridium sporogenes
Clostridium subterminale
Clostridium tertium
Clostridium tetani
Clostridium tyrobutyricum
Comamonas (Pseudomonas) acidovorans
Comamonas (Pseudomonas) testosteroni
Comamonas species
Corynebacterium accolens
Corynebacterium afermentans
Corynebacterium amycolatum
Corynebacterium aquaticum
Corynebacterium argentoratense
Corynebacterium auris
Corynebacterium bovis
Corynebacterium coyleae
Corynebacterium cystitidis
Corynebacterium diphtheriae
Corynebacterium diphtheriae biotype belfanti
Corynebacterium diphtheriae biotype gravis
Corynebacterium diphtheriae biotype intermedius
Corynebacterium diphtheriae biotype mitis
Corynebacterium flavescens
Corynebacterium glucuronolyticum
Corynebacterium glucuronolyticum-seminale
Corynebacterium group A
Corynebacterium group A-4
Corynebacterium group A-5
Corynebacterium group ANF
Corynebacterium group B
Corynebacterium group B-3
Corynebacterium group F
Corynebacterium group F-1
Corynebacterium group F-2
Corynebacterium group G
Corynebacterium group G-1
Corynebacterium group G-2
Corynebacterium group I
Corynebacterium group I-2
Corynebacterium jeikeium (group JK)
Corynebacterium kutscheri (C. murium)
Corynebacterium macginleyi
Corynebacterium minutissimum
Corynebacterium pilosum
Corynebacterium propinquum
Corynebacterium pseudodiphtheriticum
Corynebacterium pseudotuberculosis
Corynebacterium pyogenes
Corynebacterium renale
Corynebacterium renale group
Corynebacterium seminale
Corynebacterium species
Corynebacterium striatum (C. flavidum)
Corynebacterium ulcerans
Corynebacterium urealyticum (group D2)
Corynebacterium xerosis
Cryptococcus albidus
Cryptococcus ater
Cryptococcus cereanus
Cryptococcus gastricus
Cryptococcus humicolus
Cryptococcus lactativorus
Cryptococcus laurentii
Cryptococcus luteolus
Cryptococcus melibiosum
Cryptococcus neoformans
Cryptococcus species
Cryptococcus terreus
Cryptococcus uniguttulatus
Debaryomyces hansenii
Debaryomyces marama
Debaryomyces polymorphus
Debaryomyces species
Dermabacter hominis
Dermacoccus (Micrococcus) nishinomiyaensis
Dietzia species
Edwardsiella hoshinae
Edwardsiella ictaluri
Edwardsiella species
Edwardsiella tarda
Eikenella corrodens
Empedobacter brevis (Flavobacterium breve)
Enterobacter aerogenes
Enterobacter agglomerans
Enterobacter amnigenus
Enterobacter amnigenus asburiae (CDC enteric group 17)
Enterobacter amnigenus biogroup 1
Enterobacter amnigenus biogroup 2
Enterobacter asburiae
Enterobacter cancerogenus
Enterobacter cloacae
Enterobacter gergoviae
Enterobacter hormaechei
Enterobacter intermedius
Enterobacter sakazakii
Enterobacter species
Enterobacter taylorae
Enterobacter taylorae (CDC enteric group 19)
Enterococcus (Streptococcus) cecorum
Enterococcus (Streptococcus) faecalis (Group D)
Enterococcus (Streptococcus) faecium(Group D)
Enterococcus (Streptococcus) saccharolyticus
Enterococcus avium (Group D)
Enterococcus casseliflavus (Steptococcus faecium subsp. casseliflavus)
Enterococcus durans (Streptococcus faecium subsp. durans) (Group D)
Enterococcus gallinarum
Enterococcus hirae
Enterococcus malodoratus
Enterococcus mundtii
Enterococcus raffinosus
Enterococcus species
Erwinia amylovora
Erwinia carotovora
Erwinia carotovora subsp. atroseptica
Erwinia carotovora subsp. betavasculorum
Erwinia carotovora subsp. carotovora
Erwinia chrysanthemi
Erwinia cypripedii
Erwinia mallotivora

Table 15. Microorganism entified by commercial systems (continu d)

Erwinia nigrifluens
Erwinia quercina
Erwinia rhapontici
Erwinia rubrifaciens
Erwinia salicis
Erwinia species
Erysipelothrix rhusiopathiae
Erysipelothrix species
Escherichia blattae
Escherichia coli
Escherichia coli A-D
Escherichia coli O157:H7
Escherichia fergusonii
Escherichia hermannii
Escherichia species
Escherichia vulneris
Eubacterium aerofaciens
Eubacterium alactolyticum
Eubacterium lentum
Eubacterium limosum
Eubacterium species
Ewingella americana
Filobasidiella neoformans
Filobasidium floriforme
Filobasidium uniguttulatum
Flavimonas oryzihabitans
Flavobacterium gleum
Flavobacterium indologenes
Flavobacterium odoratum
Flavobacterium species
Francisella novicida
Francisella philomiragia
Francisella species
Francisella tularensis
Fusobacterium mortiferum
Fusobacterium necrogenes
Fusobacterium necrophorum
Fusobacterium nucleatum
Fusobacterium species
Fusobacterium varium
Gaffkya species
Gardnerella vaginalis
Gemella haemolysans
Gemella morbillorum
Gemella species
Geotrichum candidum
Geotrichum fermentans
Geotrichum penicillarum
Geotrichum penicillatum
Geotrichum species
Gordona species
Haemophilus aegyptius
Haemophilus aphrophilus
Haemophilus ducreyi
Haemophilus haemoglobinophilus
Haemophilus haemolyticus
Haemophilus influenzae
Haemophilus influenzae biotype I
Haemophilus influenzae biotype II
Haemophilus influenzae biotype III
Haemophilus influenzae biotype IV
Haemophilus influenzae biotype V
Haemophilus influenzae biotype VI
Haemophilus influenzae biotype VII
Haemophilus influenzae biotype VIII
Haemophilus paragallinarum
Haemophilus parahaemolyticus
Haemophilus parainfluenzae
Haemophilus parainfluenzae biotype I
Haemophilus parainfluenzae biotype II
Haemophilus parainfluenzae biotype III
Haemophilus parainfluenzae biotype IV
Haemophilus parainfluenzae biotype V
Haemophilus parainfluenzae biotype VI
Haemophilus parainfluenzae biotype VII
Haemophilus parainfluenzae biotype VIII
Haemophilus paraphrohaemolyticus
Haemophilus paraphrophilus
Haemophilus segnis
Haemophilus somnus
Haemophilus species
Hafnia alvei
Hanseniaspora guilliermondii
Hanseniaspora uvarum
Hanseniaspora valbyensis
Hansenula anomala
Hansenula holstii
Hansenula polymorpha
Helicobacter (Campylobacter) cinaedi
Helicobacter (Campylobacter) fennelliae
Helicobacter (Campylobacter) pylori
Issatchenkia orientalis
Kingella denitrificans
Kingella indologenes
Kingella kingae
Kingella species
Klebsiella ornithinolytica
Klebsiella oxytoca
Klebsiella planticola
Klebsiella pneumoniae subsp. ozaenae
Klebsiella pneumoniae subsp. pneumoniae
Klebsiella pneumoniae subsp. rhinoscleromatis
Klebsiella species
Klebsiella terrigena
Kloeckera apiculata
Kloeckera apis
Kloeckera japonica
Kloeckera species
Kluyvera ascorbata
Kluyvera cryocrescens
Kluyvera species
Kluyveromyces lactis
Kluyveromyces marxianus
Kluyveromyces thermotolerans
Kocuria (Micrococcus) kristinae
Kocuria (Micrococcus) rosea
Kocuria(Micrococcus) varians
Koserella trabulsii
Kytococcus (Micrococcus) sedentarius
Lactobacillus (Weissella) viridescens
Lactobacillus A
Lactobacillus acidophilus
Lactobacillus B
Lactobacillus brevis
Lactobacillus buchneri
Lactobacillus casei
Lactobacillus casei subsp. casei
Lactobacillus casei subsp. lactosus
Lactobacillus casei subsp. rhamnosus
Lactobacillus catenaformis
Lactobacillus cellobiosus
Lactobacillus collinoides
Lactobacillus coprophilus
Lactobacillus crispatus
Lactobacillus curvatus
Lactobacillus delbrueckii subsp. bulgaricus
Lactobacillus delbrueckii subsp. delbrueckii
Lactobacillus delbrueckii subsp. lactis
Lactobacillus fermentum
Lactobacillus fructivorans
Lactobacillus helveticus
Lactobacillus helveticus subsp. jugurti
Lactobacillus jensenii
Lactobacillus lindneri
Lactobacillus minutus
Lactobacillus paracasei subsp. paracasei
Lactobacillus pentosus
Lactobacillus plantarum
Lactobacillus salivarius
Lactobacillus salivarius var. salicinius
Lactobacillus species
Lactococcus diacitilactis
Lactococcus garvieae
Lactococcus lactis subsp. cremoris
Lactococcus lactis subsp. diacitilactis
Lactococcus lactis subsp. hordniae
Lactococcus lactis subsp. lactis
Lactococcus plantarum
Lactococcus raffinolactis
Leclercia adecarboxylata
Legionella species
Leminorella species
Leptospira species
Leptotrichia buccalis
Leuconostoc (Weissella) paramesenteroides
Leuconostoc carnosum
Leuconostoc citreum
Leuconostoc gelidum
Leuconostoc lactis
Leuconostoc mesenteroides
Leuconostoc mesenteroides subsp. cremoris
Leuconostoc mesenteroides subsp. dextranicum
Leuconostoc mesenteroides subsp. mesenteroides
Leuconostoc species
Listeria grayi
Listeria innocua
Listeria ivanovii
Listeria monocytogenes
Listeria murrayi
Listeria seeligeri
Listeria species
Listeria welshimeri
Megasphaera elsdenii
Methylobacterium mesophilicum
Metschnikowia pulcherrima
Microbacterium species
Micrococcus luteus
Micrococcus lylae
Micrococcus species
Mobiluncus curtisii
Mobiluncus mulieris
Mobiluncus species
Moellerella wisconsensis
Moraxella (Branhamella) catarrhalis
Moraxella atlantae
Moraxella bovis
Moraxella lacunata
Moraxella nonliquefaciens
Moraxella osloensis
Moraxella phenylpyruvica
Moraxella species
Morganella morganii
Morganella morganii subsp. morganii
Morganella morganii subsp. sibonii
Mycobacterium africanum
Mycobacterium asiaticum
Mycobacterium avium
Mycobacterium bovis
Mycobacterium chelonae
Mycobacterium fortuitum
Mycobacterium gordonae
Mycobacterium kansasii
Mycobacterium malmoense
Mycobacterium marinum
Mycobacterium phlei
Mycobacterium scrofulaceum
Mycobacterium smegmatis
Mycobacterium species

Table 15. Microorganisms ntified by comm rcial syst ms (continued)[1].

- Mycobacterium tuberculosis
- Mycobacterium ulcerans
- Mycobacterium xenopi
- Mycoplasma fermentans
- Mycoplasma hominis
- Mycoplasma orale
- Mycoplasma pneumoniae
- Mycoplasma species
- Myroides species
- Neisseria cinerea
- Neisseria elongata subsp. elongata
- Neisseria flava
- Neisseria flavescens
- Neisseria gonorrhoeae
- Neisseria lactamica
- Neisseria meningitidis
- Neisseria mucosa
- Neisseria perflava
- Neisseria polysaccharea
- Neisseria saprophytes
- Neisseria sicca
- Neisseria subflava
- Neisseria weaveri
- Neisseria weaveri (CDC group M5)
- Nocardia species
- Ochrobactrum anthropi
- Oerskovia species
- Oerskovia xanthineolytica
- Oligella (Moraxella) urethralis
- Oligella species
- Oligella ureolytica
- Paenibacillus alvei
- Paenibacillus macerans
- Paenibacillus polymyxa
- Pantoea agglomerans
- Pantoea ananas (Erwinia uredovora)
- Pantoea dispersa
- Pantoea species
- Pantoea stewartii
- Pasteurella (Haemophilus) avium
- Pasteurella aerogenes
- Pasteurella gallinarum
- Pasteurella haemolytica
- Pasteurella haemolyticus
- Pasteurella multocida
- Pasteurella multocida SF
- Pasteurella multocida subsp. multocida
- Pasteurella multocida subsp. septica
- Pasteurella pneumotropica
- Pasteurella species
- Pasteurella ureae
- Pediococcus acidilactici
- Pediococcus damnosus
- Pediococcus pentosaceus
- Pediococcus species
- Peptococcus niger
- Peptococcus species
- Peptostreptococcus anaerobius
- Peptostreptococcus asaccharolyticus
- Peptostreptococcus indolicus
- Peptostreptococcus magnus
- Peptostreptococcus micros
- Peptostreptococcus parvulus
- Peptostreptococcus prevotii
- Peptostreptococcus productus
- Peptostreptococcus species
- Peptostreptococcus tetradius
- Phaeococcomyces exophialiae
- Photobacterium damselae
- Pichia (Hansenula) anomala
- Pichia (Hansenula) jadinii
- Pichia (Hansenula) petersonii
- Pichia angusta (Hansenula polymorpha)
- Pichia carsonii (P. vini)
- Pichia etchellsii
- Pichia farinosa
- Pichia fermentans
- Pichia membranaefaciens
- Pichia norvegensis
- Pichia ohmeri
- Pichia spartinae
- Pichia species
- Plesiomonas shigelloides
- Porphyromonas asaccharolytica
- Porphyromonas endodontalis
- Porphyromonas gingivalis
- Porphyromonas levii
- Prevotella (Bacteroides) buccae
- Prevotella (Bacteroides) buccalis
- Prevotella (Bacteroides) corporis
- Prevotella (Bacteroides) denticola
- Prevotella (Bacteroides) loescheii
- Prevotella (Bacteroides) oralis
- Prevotella (Bacteroides)disiens
- Prevotella (Bacteroides)oris
- Prevotella bivia (Bacteroides bivius)
- Prevotella intermedia (Bacteroides intermedius)
- Prevotella melaninogenica (Bacteroides melaninogenicus)
- Prevotella ruminicola
- Propionibacterium acnes
- Propionibacterium avidum
- Propionibacterium granulosum
- Propionibacterium propionicum
- Propionibacterium species
- Proteus mirabilis
- Proteus penneri
- Proteus species
- Proteus vulgaris
- Prototheca species
- Prototheca wickerhamii
- Prototheca zopfii
- Providencia alcalifaciens
- Providencia heimbachae
- Providencia rettgeri
- Providencia rustigianii
- Providencia species
- Providencia stuartii
- Providencia stuartii urea +
- Pseudomonas (Chryseomonas) luteola
- Pseudomonas acidovorans
- Pseudomonas aeruginosa
- Pseudomonas alcaligenes
- Pseudomonas cepacia
- Pseudomonas chlororaphis (P. aureofaciens)
- Pseudomonas fluorescens
- Pseudomonas fluorescens group
- Pseudomonas mendocina
- Pseudomonas pseudoalcaligenes
- Pseudomonas putida
- Pseudomonas species
- Pseudomonas stutzeri
- Pseudomonas testosteroni
- Pseudomonas vesicularis
- Pseudoramibacter (Eubacterium) alactolyticus
- Psychrobacter (Moraxella) phenylpyruvicus
- Rahnella aquatilis
- Ralstonia (Pseudomonas, Burkholderia) pickettii
- Rhodococcus (Corynebacterium) equi
- Rhodococcus species
- Rhodosporidium toruloides
- Rhodotorula glutinis
- Rhodotorula minuta
- Rhodotorula mucilaginosa (R. rubra)
- Rhodotorula species
- Rickettsia species
- Rothia dentocariosa
- Saccharomyces cerevisiae
- Saccharomyces exiguus
- Saccharomyces kluyverii
- Saccharomyces species
- Sakaguchia dacryoides (Rhodosporidium dacryoidum)
- Salmonella arizonae
- Salmonella choleraesuis
- Salmonella enteritidis
- Salmonella gallinarum
- Salmonella paratyphi A
- Salmonella paratyphi B
- Salmonella pullorum
- Salmonella species
- Salmonella typhi
- Salmonella typhimurium
- Salmonella typhisuis
- Salmonella/Arizona
- Serratia ficaria
- Serratia fonticola
- Serratia grimesii
- Serratia liquefaciens
- Serratia marcescens
- Serratia odorifera
- Serratia odorifera type 1
- Serratia odorifera type 2
- Serratia plymuthica
- Serratia proteamaculans
- Serratia proteamaculans subsp. proteamaculans
- Serratia proteamaculans subsp. quinovora
- Serratia rubidaea
- Serratia species
- Shewanella (Pseudomonas, Alteromonas) putrefaciens
- Shigella boydii
- Shigella dysenteriae
- Shigella flexneri
- Shigella sonnei
- Shigella species
- Sphingobacterium multivorum
- Sphingobacterium species
- Sphingobacterium spiritivorum
- Sphingobacterium thalpophilum
- Sphingomonas (Pseudomonas) paucimobilis
- Sporidiobolus salmonicolor
- Sporobolomyces roseus
- Sporobolomyces salmonicolor
- Sporobolomyces species
- Staphylococcus (Peptococcus) saccharolyticus
- Staphylococcus arlettae
- Staphylococcus aureus
- Staphylococcus aureus (Coagulase-negative)
- Staphylococcus auricularis
- Staphylococcus capitis
- Staphylococcus capitis subsp. capitis
- Staphylococcus capitis subsp. ureolyticus
- Staphylococcus caprae
- Staphylococcus carnosus
- Staphylococcus caseolyticus
- Staphylococcus chromogenes
- Staphylococcus cohnii
- Staphylococcus cohnii subsp. cohnii
- Staphylococcus cohnii subsp. urealyticum
- Staphylococcus epidermidis
- Staphylococcus equorum
- Staphylococcus gallinarum
- Staphylococcus haemolyticus
- Staphylococcus hominis
- Staphylococcus hominis subsp. hominis
- Staphylococcus hominis subsp. novobiosepticus

Table 15. Micro rganisms identified by comm rcial syst ms (continued)[1].

Staphylococcus hyicus
Staphylococcus intermedius
Staphylococcus kloosii
Staphylococcus lentus
Staphylococcus lugdunensis
Staphylococcus saprophyticus
Staphylococcus schleiferi
Staphylococcus sciuri
Staphylococcus simulans
Staphylococcus species
Staphylococcus warneri
Staphylococcus xylosus
Stenotrophomonas (*Xanthomonas*) *maltophilia*
Stephanoascus ciferrii
Stomatococcus mucilaginosus
Streptococcus acidominimus
Streptococcus agalactiae
Streptococcus agalactiae (Group B)
Streptococcus agalactiae hemolytic
Streptococcus agalactiae non-h molytic
Streptococcus alactolyticus
Streptococcus anginosus
Streptococcus anginosus (Group D, nonenterococci)
Streptococcus beta-hemolytic group A
Streptococcus beta-hemolytic non-group A or B
Streptococcus beta-hemolytic non-group A
Streptococcus beta-hemolytic
Streptococcus bovis (Group *D*, nonenterococci)
Streptococcus bovis I
Streptococcus bovis II
Streptococcus canis
Streptococcus constellatus
Streptococcus constellatus (*Streptococcus milleri* I)
Streptococcus constellatus (viridans *Streptococcus*)
Streptococcus downei
Streptococcus dysgalactiae subsp. *dysgalactiae*
Streptococcus dysgalactiae subsp. *equisimilis*
Streptococcus equi (Group C/Group G *Streptococcus*)
Streptococcus equi subsp. *equi*
Streptococcus equi subsp. *zooepidemicus*
Streptococcus equinus
Streptococcus equinus (Group D, nonenterococci)
Streptococcus equisimilis
Streptococcus equisimulis (Group C/Group G *Streptococcus*)
Streptococcus Gamma (non)-hemolytic
Streptococcus gordonii
Streptococcus Group B
Streptococcus Group C
Streptococcus Group D
Streptococcus Group E
Streptococcus Group F
Streptococcus Group G
Streptococcus Group L
Streptococcus Group P
Streptococcus Group U
Streptococcus intermedius
Streptococcus intermedius (*Streptococcus milleri* II)
Streptococcus intermedius (viridans *Streptococcus*)
Streptococcus milleri group
Streptococcus mitis
Streptococcus mitis (viridans *Streptococcus*)
Streptococcus mitis group
Streptococcus mutans
Streptococcus mutans (viridans *Streptococcus*)
Streptococcus oralis
Streptococcus parasanguis
Streptococcus pneumoniae
Streptococcus porcinus
Streptococcus pyogenes
Streptococcus pyogenes (Group *A*)
Streptococcus salivarius
Streptococcus salivarius (viridans *Streptococcus*)
Streptococcus salivarius subsp. *salivarius*
Streptococcus salivarius subsp. *thermophilus*
Streptococcus sanguis
Streptococcus sanguis I (viridans *Streptococcus*)
Streptococcus sanguis II
Streptococcus sanguis II (viridans *Streptococcus*)
Streptococcus sobrinus
Streptococcus species
Streptococcus suis I
Streptococcus suis II
Streptococcus uberis
Streptococcus uberis (viridans *Streptococcus*)
Streptococcus vestibularis
Streptococcus zooepidemicus
Streptococcus zooepidemicus (Group C)
Streptomyces somaliensis
Streptomyces species
Suttonella (*Kingella*) *indologenes*
Tatumella ptyseos
Tetragenococcus (*Pediococcus*) *halophilus*
Torulaspora delbrueckii (*Saccharomyces rosei*)
Torulopsis candida
Torulopsis haemulonii
Torulopsis inconspicua
Treponema species
Trichosporon asahii
Trichosporon asteroides
Trichosporon beigelii
Trichosporon cutaneum
Trichosporon inkin
Trichosporon mucoides
Trichosporon ovoides
Trichosporon pullulans
Trichosporon species
Turicella otitidis
Ureaplasma species
Ureaplasma urealyticum
Veillonella parvula (*V. alcalescens*)
Veillonella species
Vibrio alginolyticus
Vibrio cholerae
Vibrio damsela
Vibrio fluvialis
Vibrio furnissii
Vibrio harveyi
Vibrio hollisae
Vibrio metschnikovii
Vibrio mimicus
Vibrio parahaemolyticus
Vibrio species
Vibrio species SF
Vibrio vulnificus
Weeksella (Bergeylla) virosa
Weeksella species
Weeksella virosa
Williopsis (*Hansenula*) *saturnus*
Xanthomonas campestris
Xanthomonas species
Yarrowia (*Candida*) *lipolytica*
Yersinia aldovae
Yersinia enterocolitica
Yersinia enterocolitica group
Yersinia frederiksenii
Yersinia intermedia
Yersinia intermedius
Yersinia kristensenii
Yersinia pestis
Yersinia pseudotuberculosis
Yersinia pseudotuberculosis SF
Yersinia ruckeri
Yersinia species
Yokenella regensburgei
Yokenella regensburgei (*Koserella trabulsii*)
Zygoascus hellenicus
Zygosaccharomyces species

[1] The list includes microorganisms that may be identified by API identification test systems and VITEK® automated identification system from bioMérieux Inc., or by the MicroScan® - WalkAway® automated systems from Dade Behring. Identification relies on classical identification methods using batteries of biochemical and other phenotypical tests.

Table 16. *tuf* gene sequences obtained in our laboratory (Example 42).

Species	Strain no.	Gene	GenBank Accession no.*
Abiotrophia adiacens	ATCC49175	*tuf*	AF124224
Enterococcus avium	ATCC14025	*tufA*	AF124220
		tufB	AF274715
Enterococcus casseliflavus	ATCC25788	*tufA*	AF274716
		tufB	AF274717
Enterococcus cecorum	ATCC43198	*tuf*	AF274718
Enterococcus columbae	ATCC51263	*tuf*	AF274719
Enterococcus dispar	ATCC51266	*tufA*	AF274720
		tufB	AF274721
Enterococcus durans	ATCC19432	*tufA*	AF274722
		tufB	AF274723
Enterococcus faecalis	ATCC29212	*tuf*	AF124221
Enterococcus faecium	ATCC 19434	*tufA*	AF124222
		tufB	AF274724
Enterococcus gallinarum	ATCC49573	*tufA*	AF124223
		tufB	AF274725
Enterococcus hirae	ATCC8043	*tufA*	AF274726
		tufB	AF274727
Enterococcus malodoratus	ATCC43197	*tufA*	AF274728
		tufB	AF274729
Enterococcus mundtii	ATCC43186	*tufA*	AF274730
		tufB	AF274731
Enterococcus pseudoavium	ATCC49372	*tufA*	AF274732
		tufB	AF274733
Enterococcus raffinosus	ATCC49427	*tufA*	AF274734
		tufB	AF274735
Enterococcus saccharolyticus	ATCC43076	*tuf*	AF274736
Enterococcus solitarius	ATCC49428	*tuf*	AF274737
Enterococcus sulfureus	ATCC49903	*tuf*	AF274738
Lactococcus lactis	ATCC11154	*tuf*	AF274745
Listeria monocytogenes	ATCC15313	*tuf*	AF274746
Listeria seeligeri	ATCC35967	*tuf*	AF274747
Staphylococcus aureus	ATCC25923	*tuf*	AF274739
Staphylococcus epidermidis	ATCC14990	*tuf*	AF274740
Streptococcus mutans	ATCC25175	*tuf*	AF274741
Streptococcus pneumoniae	ATCC6303	*tuf*	AF274742
Streptococcus pyogenes	ATCC19615	*tuf*	AF274743
Streptococcus suis	ATCC43765	*tuf*	AF274744

*Corresponding sequence ID NO. for the above ATCC strains are given in table 7.

Table 17. *tuf* g ne sequenc s selected from databases for Exampl 42.

Speci s	Gene	Accession no.*
Agrobacterium tumefaciens	*tufA*	X99673
	tufB	X99674
Anacystis nidulans	*tuf*	X17442
Aquifex aeolicus	*tufA*	AE000657
	tufB	AE000657
Bacillus stearothermophilus	*tuf*	AJ000260
Bacillus subtilis	*tuf*	AL009126
Bacteroides fragilis	*tuf*	P33165
Borrelia burgdorferi	*tuf*	AE000783
Brevibacterium linens	*tuf*	X76863
Bulkholderia cepacia	*tuf*	P33167
Campylobacter jejuni	*tufB*	Y17167
Chlamydia pneumoniae	*tuf*	AE001363
Chlamydia trachomatis	*tuf*	M74221
Corynebacterium glutamicum	*tuf*	X77034
Cytophaga lytica	*tuf*	X77035
Deinococcus radiodurans	*tuf*	AE000513
Escherichia coli	*tufA*	J01690
	tufB	J01717
Fervidobacterium islandicum	*tuf*	Y15788
Haemophilus influenzae	*tufA*	L42023
	tufB	L42023
Helicobacter pylori	*tuf*	AE000511
Homo sapiens (Human)	*EF-1α*	X03558
Methanococcus jannaschii	*EF-1α*	U67486
Mycobacterium leprae	*tuf*	D13869
Mycobacterium tuberculosis	*tuf*	X63539
Mycoplasma genitalium	*tuf*	L43967
Mycoplasma pneumoniae	*tuf*	U00089
Neisseria gonorrhoeae	*tufA*	L36380
Nicotiana tabacum (Tobacco)	*EF-1α*	U04632
Peptococcus niger	*tuf*	X76869
Planobispora rosea	*tuf1*	U67308
Saccharomyces cerevisiae (Yeast)	*EF-1α*	X00779
Salmonella typhimurium	*tufA*	X55116
	tufB	X55117
Shewanella putrefaciens	*tuf*	P33169
Spirochaeta aurantia	*tuf*	X76874
Spirulina platensis	*tufA*	X15646
Streptomyces aureofaciens	*tuf1*	AF007125
Streptomyces cinnamoneus	*tuf1*	X98831
Streptomyces coelicolor	*tuf1*	X77039
	tuf3	X77040
Streptomyces collinus	*tuf1*	S79408
Streptomyces ramocissimus	*tuf1*	X67057
	tuf2	X67058
	tuf3	X67059
Synechocystis sp.	*tuf*	AB001339
Taxeobacter ocellatus	*tuf*	X77036
Thermotoga maritima	*tuf*	AE000512
Thermus aquaticus	*tuf*	X66322
Thermus thermophilus	*tuf*	X06657
Thiobacillus cuprinus	*tuf*	U78300
Treponema pallidum	*tuf*	AE000520
Wolinella succinogenes	*tuf*	X76872

* Sequence data were obtained from GenBank, EMBL, and SWISSPROT databases. Genes were designated as appeared in th references.

Tabl 18. Nucl tide and amino acid s quence identities of EF-Tu b tween different nt rococci and other l w G+C gram-positive bact ria.

The upper right triangle represents the deduced amino acid sequence identities of gram-positive bacterial EF-Tu, while the lower left triangle represents the DNA sequence identities of the corresponding *tuf* genes. The sequence identities between different enterococcal *tufA* genes are boxed while those between enterococcal *tufB* genes are shaded.

Bacterial *tuf* gene	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
1. *E. avium tufA*		96	98	96	96	96	96	97	95	98	99	95	95	96	94	96	93	86	87	85	88	88	88	86	85	86	87	86	92	91	90	90	90	92	84	85	84	82	83
2. *E. casseliflavus tufA*	90		87	96	98	99	96	95	96	96	98	95	95	96	96	94	93	87	88	88	87	87	86	87	87	87	88	88	94	91	90	91	91	92	86	87	85	85	85
3. *E. dispar tufA*	93	90		95	95	96	95	96	95	97	97	91	80	85	95	95	93	86	87	85	87	87	86	87	86	87	87	87	93	90	89	90	90	92	85	86	84	85	84
4. *E. durans tufA*	90	89	90		98	96	99	93	99	95	96	90	91	94	95	94	92	87	87	86	86	86	85	86	87	87	88	87	94	90	90	90	90	91	85	86	84	84	84
5. *E. faecium tufA*	88	90	89	96		96	98	93	98	95	96	89	91	88	94	93	92	87	88	86	86	87	87	86	87	87	88	87	94	92	91	91	91	93	85	86	84	84	84
6. *E. gallinarum tufA*	90	97	89	89	89		96	93	95	96	96	88	89	89	96	93	92	87	87	86	87	87	87	86	87	87	88	87	93	92	90	90	90	93	85	86	84	83	84
7. *E. hirae tufA*	90	90	89	99	98	89		93	99	95	96	91	91	89	95	94	92	86	87	88	88	86	85	86	86	87	87	87	94	90	90	90	90	91	85	86	84	84	84
8. *E. malodoratus tufA*	96	91	94	90	89	90	89		92	97	97	89	89	90	93	96	92	86	85	82	85	85	85	85	83	85	86	86	92	90	88	88	89	91	83	84	83	83	82
9. *E. mundtii tufA*	89	89	88	96	93	89	96	88		94	95	88	90	88	94	94	92	87	87	88	86	86	85	86	87	87	88	87	94	90	89	90	89	91	85	86	84	84	84
10. *E. pseudoavium tufA*	97	92	93	90	89	91	89	97	89		98	90	90	91	95	96	94	87	87	86	87	87	86	87	86	87	88	88	93	90	89	90	90	91	85	86	85	85	84
11. *E. raffinosus tufA*	97	91	93	90	89	89	89	97	88	97		91	90	90	94	96	93	86	87	85	86	86	85	86	85	87	87	87	93	89	89	90	89	91	84	85	84	84	83
12. *E. cecorum tufA*	90	90	95	96	96	95	96	92	95	95	95		98	95	93	93	93	88	88	87	87	87	86	86	89	87	89	89	93	90	90	91	91	93	86	86	84	85	84
13. *E. columbae tufA*	90	90	95	96	97	96	96	93	95	95	95	97		95	94	92	92	89	88	88	87	88	88	87	87	87	89	89	94	92	91	91	92	93	86	86	85	88	85
14. *E. faecalis tufA*	91	91	90	89	96	97	94	94	94	95	96	90	89		94	94	93	87	87	88	87	87	86	86	87	87	88	87	93	91	89	90	91	93	86	88	86	85	85
15. *E. saccharolyticus tufA*	91	91	91	90	87	90	89	91	89	92	91	89	89	82		94	92	86	87	85	87	86	84	86	65	87	87	87	92	90	89	89	68	90	84	85	84	84	84
16. *E. sulfureus tufA*	91	89	90	91	88	88	90	91	89	92	91	88	89	91	94		91	85	84	81	84	85	84	84	81	84	85	85	91	90	87	88	89	91	82	83	83	82	82
17. *E. solitarius tuf*	83	84	83	83	84	83	82	84	83	84	84	84	83	84	83	83		88	87	86	87	87	86	87	88	88	88	89	92	91	89	90	90	91	86	85	85	85	84
18. *E. avium tufB*	77	77	78	78	76	77	78	78	77	78	77	78	78	78	77	76	77		93	93	94	94	94	92	98	93	99	97	87	86	87	86	85	86	89	88	87	85	86
19. *E. casseliflavus tufB*	71	72	72	72	70	72	72	70	71	72	72	72	70	72	72	68	72	79		93	95	95	96	95	93	95	94	94	87	86	88	88	84	85	90	90	89	88	88
20. *E. dispar tufB*	76	78	77	77	77	77	77	76	77	76	77	77	77	77	78	75	78	82	79		91	91	92	91	94	92	93	93	86	83	85	85	82	84	89	89	87	87	86
21. *E. durans tufB*	77	78	78	78	78	77	78	77	78	77	78	77	77	78	78	75	75	83	80	82		98	95	97	94	97	95	94	87	86	88	88	84	85	90	91	89	88	89
22. *E. faecium tufB*	76	75	76	76	75	77	76	76	76	75	76	77	77	77	76	74	74	80	78	79	86		96	97	95	97	95	94	87	87	88	88	84	86	90	90	89	87	87
23. *E. gallinarum tufB*	72	73	72	73	72	74	72	71	72	72	72	72	72	73	73	72	72	78	81	77	81	82		94	94	95	95	94	85	87	89	89	84	86	90	90	89	87	88
24. *E. hirae tufB*	75	74	76	75	75	75	75	75	76	76	75	74	74	74	75	72	74	80	79	79	84	83	79		93	87	93	94	87	85	86	88	83	85	89	90	88	88	87
25. *E. malodoratus tufB*	76	76	76	77	77	77	77	74	77	76	76	77	75	77	77	73	78	90	79	83	81	80	77	79		93	98	97	87	86	87	87	85	86	88	89	87	85	86
26. *E. mundtii tufB*	74	74	74	75	73	74	74	74	74	74	74	74	74	75	74	71	73	80	80	78	85	85	80	84	80		94	94	87	88	88	88	84	86	90	90	89	88	89
27. *E. pseudoavium tufB*	77	77	78	77	78	78	77	77	76	78	78	77	77	78	78	77	78	91	80	85	84	81	79	80	91	80		98	88	87	88	87	85	87	90	89	88	86	87
28. *E. raffinosus tufB*	78	79	79	78	77	77	78	78	77	79	79	78	78	78	79	77	79	90	79	84	84	81	77	80	90	81	92		87	85	87	88	84	86	90	89	88	88	87
29. *A. adiacens tuf*	88	87	87	86	88	86	86	89	86	88	88	87	88	88	88	90	82	77	70	76	77	76	71	73	77	73	78	78		90	88	89	90	91	85	86	84	85	83
30. *B. subtilis tuf*	81	80	79	79	80	80	79	79	79	80	81	80	81	81	80	78	78	73	69	73	73	71	70	71	72	71	74	74	78		91	92	90	90	82	82	83	82	84
31. *L. monocytogenes tuf*	82	81	82	82	82	82	82	81	81	81	82	81	81	81	81	79	79	76	71	76	75	75	73	74	75	73	78	76	79	82		99	88	90	84	84	84	84	84
32. *L. seeligeri tuf*	82	81	82	82	82	81	82	81	82	81	82	81	82	80	81	79	79	76	71	76	75	74	73	75	75	73	77	76	79	82	99		88	91	84	85	85	84	85
33. *S. aureus tuf*	84	84	83	83	83	84	84	82	84	83	84	86	88	84	82	81	79	75	69	75	75	73	69	72	74	72	74	74	83	79	81	81		98	81	82	82	80	82
34. *S. epidermidis tuf*	83	85	83	84	83	84	84	82	84	83	83	86	87	85	83	82	79	75	69	75	76	73	68	72	74	72	74	75	81	79	82	81	94		83	83	83	83	83
35. *S. mutans tuf*	76	77	76	76	76	77	76	75	76	76	76	77	76	76	76	74	78	79	72	77	78	77	74	75	78	75	78	81	77	75	76	77	74	73		97	96	94	88
36. *S. pneumoniae tuf*	76	77	76	77	77	77	77	75	78	76	76	77	76	77	75	74	75	76	72	76	78	76	73	74	77	75	75	78	75	76	77	76	74	74	87		96	96	89
37. *S. pyogenes tuf*	76	77	76	77	76	75	77	74	77	76	75	76	75	77	75	73	75	74	71	75	78	75	73	74	75	75	75	77	76	77	76	76	73	72	87	93		94	89
38. *S. suis tuf*	74	78	76	76	74	75	76	74	78	76	77	77	75	78	76	73	75	74	71	75	78	74	70	74	75	73	73	77	77	77	77	77	72	73	88	93	91		88
39. *L. lactis tuf*	75	76	75	76	75	75	76	75	76	76	76	77	76	76	75	72	74	75	72	75	77	76	71	75	74	75	75	75	75	75	77	76	74	74	80	83	82	81	

Table 19. Strains analyzed in Example 43.

Taxon	Strain*	Strain†	16S rDNA sequence accessi n number
Cedecea	ATCC 33431^T		
Cedecea lapagei	ATCC 33432^T		
Cedecea neteri	ATCC 33855^T		
Citrobacter amalonaticus	ATCC 25405^T	CDC 9020-77^T	AF025370
Citrobacter braakii	ATCC 43162		
		CDC 080-58^T	AF025368
Citrobacter farmeri	ATCC 51112^T	CDC 2991-81^T	AF025371
Citrobacter freundii	ATCC 8090^T	DSM 30039^T	AJ233408
Citrobacter koseri	ATCC 27156^T		
Citrobacter sedlakii	ATCC 51115^T	CDC 4696-86^T	AF025364
Citrobacter werkmanii	ATCC 51114^T	CDC 0876-58^T	AF025373
Citrobacter youngae	ATCC 29935^T		
Edwardsiella hoshinae	ATCC 33379^T		
Edwardsiella tarda	ATCC 15947^T		
		CDC 4411-68	AF015259
Enterobacter aerogenes	ATCC 13048^T	JCM 1235^T	AB004750
Enterobacter agglomerans	ATCC 27989		
Enterobacter amnigenus	ATCC 33072^T	JCM 1237^T	AB004749
Enterobacter asburiae	ATCC 35953^T	JCM 6051^T	AB004744
Enterobacter cancerogenus	ATCC 35317^T		
Enterobacter cloacae	ATCC 13047^T		
Enterobacter gergoviae	ATCC 33028^T	JCM 1234^T	AB004748
Enterobacter hormaechei	ATCC 49162^T		
Enterobacter sakazakii	ATCC 29544^T	JCM 1233^T	AB004746
Escherichia coli	ATCC 11775^T	ATCC 11775^T	X80725
Escherichia coli	ATCC 25922	ATCC 25922	X80724
Escherichia coli (ETEC)	ATCC 35401		
Escherichia coli (O157:H7)	ATCC 43895	ATCC 43895	Z83205
Escherichia fergusonii	ATCC 35469^T		
Escherichia hermanii	ATCC 33650^T		
Escherichia vulneris	ATCC 33821^T	ATCC 33821^T	X80734
Ewingella americana	ATCC 33852^T		
		NCPPB 3905	X88848
Hafnia alvei	ATCC 13337^T	ATCC 13337^T	M59155
Klebsiella ornithinolytica	ATCC 31898		
		CIP 103.364	U78182
Klebsiella oxytoca	ATCC 33496		
		ATCC 13182^T	U78183
Klebsiella planticola	ATCC 33531^T	JCM 7251^T	AB004755
Klebsiella pneumoniae			
subsp. *pneumoniae*	ATCC 13883^T	DSM 30104^T	AJ233420
subsp. *ozaenae*	ATCC 11296^T	ATCC 11296^T	Y17654
subsp. *rhinoscleromatis*	ATCC 13884^T		

Tabl 19. Strains analyz d in Exampl 43 (continued).

Taxon	Strain*	Strain†	16S rDNA s quence accessi n number
Kluyvera ascorbata	ATCC 33433^T		
		ATCC 14236	Y07650
Kluyvera cryocrescens	ATCC 33435^T		
Kluyvera georgiana	ATCC 51603^T		
Leclercia adecarboxylata	ATCC 23216^T		
Leminorella grimontii	ATCC 33999^T	DSM 5078^T	AJ233421
Moellerella wisconsensis	ATCC 35017^T		
Morganella morganii	ATCC 25830^T		
Pantoea agglomerans	ATCC 27155^T	DSM 3493^T	AJ233423
Pantoea dispersa	ATCC 14589^T		
Plesiomonas shigelloïdes	ATCC 14029^T		
Pragia fontium	ATCC 49100^T	DSM 5563^T	AJ233424
Proteus mirabilis	ATCC 25933		
Proteus penneri	ATCC 33519^T		
Proteus vulgaris	ATCC 13315^T	DSM 30118^T	AJ233425
Providencia alcalifaciens	ATCC 9886^T		
Providencia rettgeri	ATCC 9250		
Providencia rustigianii	ATCC 33673^T		
Providencia stuartii	ATCC 33672		
Rahnella aquatilis	ATCC 33071^T	DSM 4594^T	AJ233426
Salmonella choleraesuis			
subsp. *arizonae*	ATCC 13314^T		
subsp. *choleraesuis*			
serotype Choleraesuis	ATCC 7001		
serotype Enteritidis‡	ATCC 13076^T		
		SE22	SE22
serotype Gallinarum	ATCC 9184		
serotype Heidelberg	ATCC 8326		
serotype Paratyphi A	ATCC 9150		
serotype Paratyphi B	ATCC 8759		
serotype Typhi‡	ATCC 10749		
		St111	U88545
serotype Typhimurium‡	ATCC 14028		
serotype Virchow	ATCC 51955		
subsp. *diarizonae*	ATCC 43973^T		
subsp. *houtenae*	ATCC 43974^T		
subsp. *indica*	ATCC 43976^T		
subsp. *salamae*	ATCC 43972^T		
Serratia fonticola	DSM 4576^T	DSM 4576^T	AJ233429
Serratia grimesii	ATCC 14460^T	DSM 30063^T	AJ233430
Serratia liquefaciens	ATCC 27592^T		
Serratia marcescens	ATCC 13880^T	DSM 30121^T	AJ233431
Serratia odorifera	ATCC 33077^T	DSM 4582^T	AJ233432
Serratia plymuthica	DSM 4540^T	DSM 4540^T	AJ233433
Serratia rubidaea	DSM 4480^T	DSM 4480^T	AJ233436
Shigella boydii	ATCC 9207	ATCC 9207	X96965
Shigella dysenteriae	ATCC 11835		
		ATCC 13313^T	X96966
		ATCC 25931	X96964

Tabl 19. Strains analyz d in Exampl 43 (c ntinued).

Taxon	Strain*	Strain†	16S rDNA sequence acc ssion number
Shigella flexneri	ATCC 12022	ATCC 12022	X96963
Shigella sonnei	ATCC 29930^T		
Tatumella ptyseos	ATCC 33301^T	DSM 5000^T	AJ233437
Trabulsiella guamensis	ATCC 49490^T		
Yersinia enterocolitica	ATCC 9610^T	ATCC 9610^T	M59292
Yersinia frederiksenii	ATCC 33641^T		
Yersinia intermedia	ATCC 29909^T		
Yersinia pestis	RRB KIMD27		
		ATCC 19428^T	X75274
Yersinia pseudotuberculosis	ATCC 29833^T		
Yersinia rohdei	ATCC 43380^T	ER-2935^T	X75276
Shewanella putrefaciens	ATCC 8071^T		
Vibrio cholerae	ATCC 25870		
		ATCC.14035^T	X74695

T Type strain
*Strains used in this study for sequencing of partial *tuf* and *atpD* genes. SEQ ID NOs. for *tuf* and *atpD* sequences corresponding to the above reference strains are given in table 7.
†Strains used in other studies for sequencing of 16S rDNA gene. When both strain numbers are on the same row, both strains are considered to be the same although strain numbers may be different.
‡Phylogenetic serotypes considered species by the Bacteriological Code (1990 Revision).

Table 20. PCR primer pairs used in this study

Prim r SEQ ID NO.	Sequence	Nucl otide positions*	Amplic n length (bp)
tuf			
664	5'-AAYATGATIACIGGIGCIGCICARATGGA-3'	271-299	884
697	5'-CCIACIGTICKICCRCCYTCRCG-3'	1132-1156	
atpD			
568	5'-RTIATIGGIGCIGTIRTIGAYGT-3'	25-47	884
567	5'-TCRTCIGCIGGIACRTAIAYIGCYTG-3'	883-908	
700	5'-TIRTIGAYGTCGARTTCCCTCARG-3'	38-61	871
567	5'-TCRTCIGCIGGIACRTAIAYIGCYTG-3'	883-908	

*The nucleotide positions given are for *E. coli tuf* and *atpD* sequences (GenBank accession no. AE000410 and V00267, respectively). Numbering starts from the first base of the initiation codon.

Table 21. Selection f *M. catarrhalis*-specific primer pairs from SEQ ID NO: 29[1] (466 pb DNA fragment) other than those previously tested[2].

Primer	Sequence	Amplicon size (bp)	*Moraxella catarrhalis* ATCC 43628	*Moraxella catarrhalis* ATCC 53879	*Moraxella nonliquefaciens*	*Moraxella lacunata*	*Moraxella osloensis*	*Moraxella atlantae*	*Moraxella phenylpyruvica*	*Kingella indologenes*	*Kingella kingea*	*Neisseria meningitidis*	*Neisseria gonorrhoeae*	*Escherichia coli*	*Staphylococcus aureus*
SEQ ID NO:118	CGCTGACGGCTTGTTTGTACCA	118	+[3]	+	-	-	-	-	-	-	-	-	-	-	-
SEQ ID NO:119	TGTTTTGAGCTTTTTATTTTTTGA														
VBmcat1	TGCTTAAGATTCACTCTGCCATTTT	93	+	+	-	-	-	-	-	-	-	-	-	-	-
VBmcat2	TAAGTCGCTGACGGCTTGTTT														
VBmcat3	CCTGCACCACAAGTCATCAT	140	+	+	-	-	-	-	-	-	-	-	-	-	-
VBmcat4	AATTCACCAACAATGTCAAAGC														
VBmcat5	AATGATAACCAGTCAAGCAAGC	219	+	+	-	-	-	-	-	-	-	-	-	-	-
VBmcat6	GGTGCATGGTGATTTGTAAAA														
VBmcat7	GTGTGCGTTCACTTTTACAAAT	160	+	+	-	-	-	-	-	-	-	-	-	-	-
VBmcat8	GGTGTTAAGCTGATGATGAGAG														
VBmcat9	TGACCATGCACACCCTTATT	167	+	+	-	-	-	-	-	-	-	-	-	-	-
VBmcat10	TCATTGGGATGAAAGTATCGTT														

[1] SEQ ID NO. from US patent 6,001,564.

[2] All PCR assays were performed with 1 ng of purified genomic DNA by using an annealing temperature of 55°C and 30 cycles of amplification. The genomic DNA from the various bacterial species above was always isolated from reference strains obtained from ATCC.

[3] All positive results showed a strong amplification signal with genomic DNA from the target species *M. catarrhalis*.

Table 22. Selection of *S. epidermidis*-specific primer pairs from SEQ ID NO: 36[1] (705 pb DNA fragment) other than those previously tested.

Primer	Sequence (all 25 nucleotides)	Amplicon size (bp)	*Staphylococcus epidermidis* ATCC 14990	*Staphylococcus epidermidis* ATCC 12228	*Staphylococcus capitis*	*Staphylococcus cohnii*	*Staphylococcus aureus*	*Staphylococcus auricularis*	*Staphylococcus*	*Staphylococcus hominis*	*Staphylococcus*	*Staphylococcus*	*Staphylococcus simulans*	*Staphylococcus warneri*	*Bacillus subtilis*	*Enterococcus faecalis*	*Enterococcus faecium*	*Enterococcus gallinarum*	*Listeria monocytogenes*	*Streptococcus agalactiae*	*Streptococcus pneumoniae*	*Streptococcus pyogenes*	Annealing temperature [2] (°C)
SEQ ID NO:145	ATCAAAAAGTTGGCGAACCTTTTCA	125	+[3]	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	55
SEQ ID NO:146	CAAAAGAGCGTGGAGAAAAGTATCA																						
VBsep3	CATAGTCTGATTGCTCAAAGTCTTG	208	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	55
VBsep4	GCGAATAGTGAACTACATTCTGTTG		+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	60
VBsep5	CACGCTCTTTTGCAATTTCCATTGA	208	+	+	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	55
VBsep6	GAAGCAAATATTCAAAATGCACCAG		+	+	+	+	+	-	+	+	-	-	-	NT	NT	NT	NT	NT	NT	NT	NT	NT	65
VBsep7	AAAGTCTTTTGCTTCTTCAGATTCA	177	+	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	55
VBsep8	GTGTTCACAGGTATGGATGCTCTTA		+	+	NT	NT	-	NT	-	NT	-	-	+	NT	NT	NT	NT	NT	NT	NT	NT	NT	60
			+	+	NT	NT	-	NT	-	NT	-	-	-	NT	NT	NT	NT	NT	NT	NT	NT	NT	65
VBsep9	GAGCATCCATACCTGTGAACACAGA	153	+	+	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	55
VBsep10	TTTTCCAATTACAAGAGACATCAGT		+	+	NT	NT	-	NT	+	NT	+	-	-	NT	NT	NT	NT	NT	NT	NT	NT	NT	60
			+	+	NT	NT	-	NT	-	NT	-	-	-	NT	NT	NT	NT	NT	NT	NT	NT	NT	65
VBsep11	TTTGAATTCGCATGTACTTTGTTTG	135	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	55
VBsep12	CCCCGGGTTCGAAATCGATAAAAAG																						

[1] SEQ ID NO. from US patent 6,001,564.

[2] All PCR assays were performed with 1 ng of purified genomic DNA by using an annealing temperature of 55 to 65°C and 30 cycles of amplification. The genomic DNA from the various bacterial species above was always isolated from reference strains obtained from ATCC.

[3] All positive results showed a strong amplification signal with genomic DNA from the target species *S. epidermidis*. The instensity of the positive amplification signal with species other than *S. epidermidis* was variable.

NT = not tested.

Table 23. **Influence of nucleotide variation(s) on the efficiency of the PCR amplification: Example with SEQ ID NO: 146 from *S. epidermidis*.**

Primer[1]	Sequence (all 25 nucleotides)	Number of mutation	*Staphyloccus epidermidis*[2] ATCC 14990				*Staphylococcus aureus*[3]
			50°C	55°C			50°C
			1	1	0,1	0,01	1
SEQ ID NO:145	ATCAAAAAGTTGGCGAACCTTTTCA	0					
SEQ ID NO:146	CAAAAGAGCGTGGAGAAAAGTATCA	0	3+[4]	3+	2+	+	-
VBmut1	CAAAAGAGCGTGGAGAAAAGTACCA	1	3+	3+	2+	+	-
VBmut2	CAAAAGAGCGTGGAGAAAAATATCA	1	3+	3+	2+	+	-
VBmut3	CAAAAGAGCGTGGAGAGAAGTATCA	1	3+	3+	2+	+	-
VBmut4	CAAAAGAGCGTGGTGAAAAGTATCA	1	3+	3+	2+	+	-
VBmut5	CAAAAGAGCGCGGAGAAAAGTATCA	1	3+	3+	2+	+	-
VBmut6	CAAAAGAACGTGGAGAAAAGTATCA	1	3+	3+	2+	+	-
VBmut7	CAAAGGAGCGTGGAGAAAAGTATCA	1	3+	3+	2+	+	-
VBmut8	CTAAAGAGCGTGGAGAAAAGTATCA	1	3+	3+	2+	+	-
VBmut9	CAAAAGAGCGTGGAGAGAAGTACCA	2	3+	3+	2+	+	-
VBmut10	CAAAAGAGCGCGGAGAGAAGTATCA	2	3+	3+	2+	+	-
VBmut11	CAAAGGAGCGCGGAGAAAAGTATCA	2	3+	3+	2+	+	-
VBmut12	CAAAGGAGCGTGGTGAAAAGTACCA	3	3+	3+	2+	+	-
VBmut13	CAAAGGAGCGCGGAGAGAAGTACCA	4	3+	2+	+	-	-

[1] All PCR tests were performed with SEQ ID NO:145 without modification combined with SEQ ID NO:146 or 13 modified versions of SEQ ID NO:146. Boxed nucleotides indicate changes in SEQ ID NO:146. All SEQ ID NOs. are from US patent 6,001,564.

[2] The tests with *S. epidermidis* were performed by using an annealing temperature of 55°C with 1, 0,1 and 0,01 ng of purified genomic DNA or at 50°C with 1 ng of purified genomic DNA.

[3] The tests with *S. aureus* were performed only at 50°C with 1 ng of genomic DNA.

[4] The intensity of the positive amplification signal was quantified as follows: 3+ = strong signal, 2+ = intermediate signal and + = weak signal.

Table 24. **Effect of the primer length on the efficiency of the PCR amplification[1]: Example with the AT-rich SEQ ID NO: 145[2] and SEQ ID NO: 146[2] from *S. epidermidis*.**

Primer	Sequence	Length (nt)	*Staphylococcus epidermidis*[3] ATCC 14990						*Staphylococcus aureus*[4]		*Staphylococcus haemolyticus*		*Staphylococcus capitis*		*Staphylococcus warneri*	
			45°C			55°C										
			1	0,1	0,01	1	0,1	0,01	45	55	45	55	45	55	45	55
VBsep301	ATATCATCAAAAAGTTGGCGAACCTTTTCA	30	NT	NT	NT	4+	3+	2+	NT	-	NT	-	NT	-	NT	-
VBsep302	AATTGCAAAAGAGCGTGGAGAAAAGTATCA	30														
SEQ ID NO:145	ATCAAAAAGTTGGCGAACCTTTTCA	25	4+[5]	3+	2+	4+	3+	2+	-	-	-	-	+	-	-	-
SEQ ID NO:146	CAAAAGAGCGTGGAGAAAAGTATCA	25														
VBsep201	AAAGTTGGCGAACCTTTTCA	20	NT	NT	NT	4+	3+	2+	NT	-	NT	-	NT	-	NT	-
VBsep202	GAGCGTGGAGAAAAGTATCA	20														
VBsep171	GTTGGCGAACCTTTTCA	17	4+	3+	2+	3+	2+	+	-	-	-	-	-	-	-	-
VBsep172	CGTGGAGAAAAGTATCA	17														
VBsep151	TGGCGAACCTTTTCA	15	3+	2+	+	-	-	-	-	-	-	-	-	-	-	-
VBsep152	TGGAGAAAAGTATCA	15														

[1] All PCR tests were performed using an annealing temperature of 45 or 55°C and 30 cycles of amplification.

[2] All SEQ ID NOs. in this Table are from US patent 6,001,546.

[3] The tests with *S. epidermidis* were made with 1, 0,1 and 0,01 ng of purified genomic DNA.

[4] The tests with all other bacterial species were made only with 1 ng of purified genomic DNA.

[5] The intensity of the positive amplification signal was quantified as follows: 4+ = very strong signal, 3+ = strong signal, 2+ = intermediate signal and + = weak signal.

NT = not tested.

Table 25. Effect of the primer length on the efficiency of the PCR amplification[1]: Example with the GC-rich SEQ ID NO: 83[2] and SEQ ID NO: 84[2] from *P. aeruginosa*.

Primer	Sequence	Length (nt)	*Pseudomonas aeruginosa*[3] ATCC 35554			*Pseudomonas fluorescens*[4]	*Burkholderia cepacia*	*Shewanella putida*	*Stenotrophomonas maltophilia*	*Neisseria meningitidis*	*Haemophilus parahaemolyticus*
			1	0,1	0,01						
SEQ ID NO 83	CGAGCGGGTGGTGTTCATC	19	2+[5]	+	-	-	-	-	-	-	-
SEQ ID NO 84	CAAGTCGTCGTCGGAGGGA	19									
Pse554-16a	CGAGCGGGTGGTGTTC	16	2+	+	-	-	-	-	-	-	-
Pse674-16a	GTCGTCGTCGGAGGGA	16									
Pse554-13b	GCGGGTGGTGTTC	13	2+	+	-	-	-	-	-	-	-
Pse674-13a	GTCGTCGGAGGGA	13									

[1] All PCR tests were performed using an annealing temperature of 55°C and 30 cycles of amplification.

[2] All SEQ ID NOs. in this Table are from US patent 6,001,546.

[3] The tests with *P. aeruginosa* were made with 1, 0,1 and 0,01 ng of purified genomic DNA.

[4] The tests with all other bacterial species were made only with 1 ng of purified genomic DNA.

[5] The intensity of the positive amplification signal was quantified as follows: 2+ = strong signal and + = moderately strong signal.

Annex I: Specific and ubiquitous primers for nucleic acid amplification (*tuf* sequences).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Bacterial species:	***Acinetobacter baumannii***		
1692	5'-GGT GAG AAC TGT GGT ATC TTA CTT	1	478-501
1693[a]	5'-CAT TTC AAC GCC TTC TTT CAA CTG	1	691-714
Bacterial species:	***Chlamydia pneumoniae***		
630	5'-CGG AGC TAT CCT AGT CGT TTC A	20	2-23
629[a]	5'-AAG TTC CAT CTC AAC AAG GTC AAT A	20	146-170
2085	5'-CAA ACT AAA GAA CAT ATC TTG CTA	20	45-68
2086[a]	5'-ATA TAA TTT GCA TCA CCT TCA AG	20	237-259
2087	5'-TCA GCT CGT GGG ATT AGG AGA G	20	431-452
2088[a]	5'-AGG CTT CAC GCT GTT AGG CTG A	20	584-605
Bacterial species:	***Chlamydia trachomatis***		
554	5'-GTT CCT TAC ATC GTT GTT TTT CTC	22	82-105
555[a]	5'-TCT CGA ACT TTC TCT ATG TAT GCA	22	249-272
Parasitical species:	***Cryptosporidium parvum***		
798	5'-TGG TTG TCC CAG CCG ATC GTT T	865	158-179
804[a]	5'-CCT GGG ACG GCC TCT GGC AT	865	664-683
799	5'-ACC TGT GAA TAC AAG CAA TCT	865	280-300
805[a]	5'-CTC TTG TCC ATC TTA GCA GT	865	895-914
800	5'-GAT GAA ATC TTC AAC GAA GTT GAT	865	307-330
806[a]	5'-AGC ATC ACC AGA CTT GAT AAG	865	946-966
801	5'-ACA ACA CCG AGA AGA TCC CA	865	353-372
803[a]	5'-ACT TCA GTG GTA ACA CCA GC	865	616-635
802	5'-TTG CCA TTT CTG GTT TCG TT	865	377-396
807[a]	5'-AAA GTG GCT TCA AAG GTT GC	865	981-1000
Bacterial species:	***Enterococcus faecium***		
1696	5'-ATG TTC CTG TAG TTG CTG GA	64	189-208
1697[a]	5'-TTT CTT CAG CAA TAC CAA CAA C	64	422-443
Bacterial species:	***Klebsiella pneumoniae***		
1329	5'-TGT AGA GCG CGG TAT CAT CAA AGT A	103	352-377
1330[a]	5'-AGA TTC GAA CTT GGT GTG CGG G	103	559-571

[a] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex I: Specific and ubiquitous primers for nucleic acid amplification (*tuf* sequences) (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Bacterial species:	***Mycoplasma pneumoniae***		
2093	5'-TGT TGG CAA TCG AAG ACA CC	2097[a]	635-654
2094[b]	5'-TTC AAT TTC TTG ACC TAC TTT CAA	2097[a]	709-732
Bacterial species:	***Neisseria gonorrhoeae***		
551	5'-GAA GAA AAA ATC TTC GAA CTG GCT A	126	256-280
552[b]	5'-TAC ACG GCC GGT GAC TAC G	126	378-396
2173	5'-AAG AAA AAA TCT TCG AAC TGG CTA	126	257-280
2174[b]	5'-TCT ACA CGG CCG GTG	126	384-398
2175	5'-CCG CCA TAC CCC GTT T	126	654-669
2176[b]	5'-CGG CAT TAC CAT TTC CAC ACC TTT	126	736-759
Bacterial species:	***Pseudomonas aeruginosa***		
1694	5'-AAG GCA AGG ATG ACA ACG GC	153	231-250
1695[b]	5'-ACG ATT TCC ACT TCT TCC TGG	153	418-438
Bacterial species:	***Streptococcus agalactiae***		
549	5'-GAA CGT GAT ACT GAC AAA CCT TTA	207-210[c]	308-331[d]
550[b]	5'-GAA GAA GAA CAC CAA CGT TG	207-210[c]	520-539[d]
Bacterial species:	***Streptococcus pyogenes***		
999	5'-TTG ACC TTG TTG ATG ACG AAG AG	1002	143-165
1000[b]	5'-TTA GTG TGT GGG TTG ATT GAA CT	1002	622-644
1001	5'-AAG AGT TGC TTG AAT TAG TTG AG	1002	161-183
1000[b]	5'-TTA GTG TGT GGG TTG ATT GAA CT	1002	622-644
Parasitical species:	***Trypanosoma brucei***		
820	5'-GAA GGA GGT GTC TGC TTA CAC	864	513-533
821[b]	5'-GGC GCA AAC GTC ACC ACA TCA	864	789-809
820	5'-GAA GGA GGT GTC TGC TTA CAC	864	513-533
822[b]	5'-CGG CGG ATG TCC TTA ACA GAA	864	909-929

[a] Sequence from databases.

[b] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

[c] These sequences were aligned to derive the corresponding primer.

[d] The nucleotide positions refer to the *S. agalactiae tuf* sequence fragment (SEQ ID NO. 209).

Annex I: Specific and ubiquitous primers for nucleic acid amplification (*tuf* sequences) (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Parasitical species:	***Trypanosoma cruzi***		
794	5'-GAC GAC AAG TCG GTG AAC TT	840-842[a]	281-300[c]
795[b]	5'-ACT TGC ACG CGA TGT GGC AG	840-842[a]	874-893[c]
Bacterial genus:	***Clostridium* sp.**		
796	5'-GGT CCA ATG CCW CAA ACW AGA	32,719-724,736[a]	32-52[d]
797[b]	5'-CAT TAA GAA TGG YTT ATC TGT SKC TCT	32,719-724,736[a]	320-346[d]
808	5'-GCI TTA IWR GCA TTA GAA RAY CCA	32,719-724,736[a]	224-247[d]
809[b]	5'-TCT TCC TGT WGC AAC TGT TCC TCT	32,719-724,736[a]	337-360[d]
810	5'-AGA GMW ACA GAT AAR SCA TTC TTA	32,719-724,736[a]	320-343[d]
811[b]	5'-TRA ART AGA ATT GTG GTC TRT ATC C	32,719-724,736[a]	686-710[d]
Bacterial genus:	***Corynebacterium* sp.**		
545	5'-TAC ATC CTB GTY GCI CTI AAC AAG TG	34-44,662[a]	89-114[e]
546[b]	5'-CCR CGI CCG GTR ATG GTG AAG AT	34-44,662[a]	350-372[e]
Bacterial genus:	***Enterococcus* sp.**		
656	5'-AAT TAA TGG CTG CAG TTG AYG A	58-72[a]	273-294[f]
657[b]	5'-TTG TCC ACG TTC GAT RTC TTC A	58-72[a]	556-577[f]
656	5'-AAT TAA TGG CTG CAG TTG AYG A	58-72[a]	273-294[f]
271[b]	5'-TTG TCC ACG TTG GAT RTC TTC A	58-72[a]	556-577[f]
1137	5'-AAT TAA TGG CTG CWG TTG AYG AA	58-72[a]	273-295[f]
1136[b]	5'-ACT TGT CCA CGT TSG ATR TCT	58-72[a]	559-579[f]

[a] These sequences were aligned to derive the corresponding primer.

[b] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

[c] The nucleotide positions refer to the *T. cruzi tuf* sequence fragment (SEQ ID NO. 842).

[d] The nucleotide positions refer to the *C. perfringens tuf* sequence fragment (SEQ ID NO. 32).

[e] The nucleotide positions refer to the *C. diphtheriae tuf* sequence fragment (SEQ ID NO. 662).

[f] The nucleotide positions refer to the *E. durans tuf* sequence fragment (SEQ ID NO. 61).

Annex I: Sp cific and ubiquitous primers for nucleic acid amplification (*tuf* sequences) (continued).

		Originating DNA fragment	
SEQ ID NO.	Nucleotide sequence	SEQ ID NO.	Nucleotide position
<u>Bacterial genus</u>:	***Legionella* sp.**		
2081	5'-GRA TYR TYA AAG TTG GTG AGG AAG	111-112[a]	411-434[b]
2082[c]	5'-CMA CTT CAT CYC GCT TCG TAC C	111-112[a]	548-569[b]
<u>Bacterial genus</u>:	***Staphylococcus* sp.**		
553	5'-GGC CGT GTT GAA CGT GGT CAA ATC A	176-203[a]	313-337[d]
575[c]	5'-TIA CCA TTT CAG TAC CTT CTG GTA A	176-203[a]	653-677[d]
553	5'-GGC CGT GTT GAA CGT GGT CAA ATC A	176-203[a]	313-337d
707[c]	5'-TWA CCA TTT CAG TAC CTT CTG GTA A	176-203[a]	653-677[d]
<u>Bacterial genus</u>:	***Streptococcus* sp.**		
547	5'-GTA CAG TTG CTT CAG GAC GTA TC	206-231[a]	372-394[e]
548[c]	5'-ACG TTC GAT TTC ATC ACG TTG	206-231[a]	548-568[e]
<u>Fungal genus</u>:	***Candida* sp.**		
576	5'-AAC TTC RTC AAG AAG GTY GGT TAC AA	407-426, 428-432[a]	332-357[f]
632[c]	5'-CCC TTT GGT GGR TCS TKC TTG GA	407-426, 428-432[a]	791-813[f]
631	5'-CAG ACC AAC YGA IAA RCC ATT RAG AT	407-426, 428-432[a]	523-548[f]
632[c]	5'-CCC TTT GGT GGR TCS TKC TTG GA	407-426, 428-432[a]	791-813[f]
633	5'-CAG ACC AAC YGA IAA RCC ITT RAG AT	407-426, 428-432[a]	523-548[f]
632[c]	5'-CCC TTT GGT GGR TCS TKC TTG GA	407-426, 428-432[a]	791-813[f]

[a] These sequences were aligned to derive the corresponding primer.

[b] The nucleotide positions refer to the *L. pneumophila tuf* sequence fragment (SEQ ID NO. 112).

[c] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

[d] The nucleotide positions refer to the *S. aureus tuf* sequence fragment (SEQ ID NO. 179).

[e] The nucleotide positions refer to the *S. agalactiae tuf* sequence fragment (SEQ ID NO. 209).

[f] The nucleotide positions refer to the *C. albicans tuf*(EF-1) sequence fragment (SEQ ID NO. 408).

Annex I: Specific and ubiquitous primers for nucleic acid amplification (*tuf* sequences) (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
<u>Fungal genus</u>:	***Cryptococcus* sp.**		
1971	5'-CYG ACT GYG CCA TCC TYA TCA	434,623,1281, 1985,1986[a]	150-170[b]
1973[c]	5'-RAC ACC RGI YTT GGW ITC CTT	434,623,1281, 1985,1986[a]	464-484[b]
1972	5'-MGI CAG CTC ATY ITT GCW KSC	434,623,1281, 1985,1986[a]	260-280[b]
1973[c]	5'-RAC ACC RGI YTT GGW ITC CTT	434,623,1281, 1985,1986[a]	464-484[b]
<u>Parasitical genus</u>:	***Entamoeba* sp.**		
703	5'-TAT GGA AAT TCG AAA CAT CT	512	38-57
704[c]	5'-AGT GCT CCA ATT AAT GTT GG	512	442-461
703	5'-TAT GGA AAT TCG AAA CAT CT	512	38-57
705[c]	5'-GTA CAG TTC CAA TAC CTG AA	512	534-553
703	5'-TAT GGA AAT TCG AAA CAT CT	512	38-57
706[c]	5'-TGA AAT CTT CAC ATC CAA CA	512	768-787
793	5'-TTA TTG TTG CTG CTG GTA CT	512	149-168
704[c]	5'-AGT GCT CCA ATT AAT GTT GG	512	442-461
<u>Parasitical genus</u>:	***Giardia* sp.**		
816	5'-GCT ACG ACG AGA TCA AGG GC	513	305-324
819[c]	5'-TCG AGC TTC TGG AGG AAG AG	513	895-914
817	5'-TGG AAG AAG GCC GAG GAG TT	513	355-374
818[c]	5'-AGC CGG GCT GGA TCT TCT TC	513	825-844
<u>Parasitical genus</u>:	***Leishmania* sp.**		
701	5'-GTG TTC ACG ATC ATC GAT GCG	514-526[a]	94-114[d]
702[c]	5'-CTC TCG ATA TCC GCG AAG CG	514-526[a]	913-932[d]

[a] These sequences were aligned to derive the corresponding primer.
[b] The nucleotide positions refer to the *C. neoformans tuf* (EF-1) sequence fragment (SEQ ID NO. 623).
[c] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.
[d] The nucleotide positions refer to the *L. tropica tuf*(EF-1) sequence fragment (SEQ ID NO. 526).

Annex I: Specific and ubiquitous prim rs for nucleic acid amplification (*tuf* sequences) (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Parasitical genus:	***Trypanosoma* sp.**		
823	5'-GAG CGG TAT GAY GAG ATT GT	529,840-842,864[a]	493-512[b]
824[c]	5'-GGC TTC TGC GGC ACC ATG CG	529,840-842,864[a]	1171-1190[b]
Bacterial family:	***Enterobacteriaceae***		
933	5'-CAT CAT CGT ITT CMT GAA CAA RTG	78,103,146,168,238,698[a]	390-413[d]
934[c]	5'-TCA CGY TTR RTA CCA CGC AGI AGA	78,103,146,168,238,698[a]	831-854[d]
Bacterial family:	***Mycobacteriaceae***		
539	5'-CCI TAC ATC CTB GTY GCI CTI AAC AAG	122	85-111
540[c]	5'-GGD GCI TCY TCR TCG WAI TCC TG	122	181-203
Bacterial group:	***Escherichia coli* and *Shigella***		
1661	5'-TGG GAA GCG AAA ATC CTG	1668[e]	283-300
1665[c]	5'-CAG TAC AGG TAG ACT TCT G	1668[e]	484-502
Bacterial group:	**Pseudomonads group**		
541	5'-GTK GAA ATG TTC CGC AAG CTG CT	153-155[a]	476-498[f]
542[c]	5'-CGG AAR TAG AAC TGS GGA CGG TAG	153-155[a]	679-702[f]
541	5'-GTK GAA ATG TTC CGC AAG CTG CT	153-155[a]	476-498[f]
544[c]	5'-AYG TTG TCG CCM GGC ATT MCC AT	153-155[a]	749-771[f]

a These sequences were aligned to derive the corresponding primer.

b The nucleotide positions refer to the *T. brucei tuf* (EF-1) sequence fragment (SEQ ID NO. 864).

c These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

d The nucleotide positions refer to the *E. coli tuf* sequence fragment (SEQ ID NO. 698).

e Sequence from databases.

f The nucleotide positions refer to the *P. aeruginosa tuf* sequence fragment (SEQ ID NO. 153).

Annex I: Specific and ubiquitous primers for nucleic acid amplification (*tuf* sequences) (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment	
		SEQ ID NO.	Nucleotide position
Parasitical group:	***Trypanosomatidae* family**		
923	5'-GAC GCI GCC ATC CTG ATG ATC	511,514-526, 529,840-842, 864[a]	166-188[b]
924[c]	5'-ACC TCA GTC GTC ACG TTG GCG	511,514-526, 529,840-842, 864[a]	648-668[b]
925	5'-AAG CAG ATG GTT GTG TGC TG	511,514-526, 529,840-842, 864[a]	274-293[b]
926[c]	5'-CAG CTG CTC GTG GTG CAT CTC GAT	511,514-526, 529,840-842, 864[a]	676-699[b]
927	5'-ACG CGG AGA AGG TGC GCT T	511,514-526, 529,840-842, 864[a]	389-407[b]
928[c]	5'-GGT CGT TCT TCG AGT CAC CGC A	511,514-526, 529,840-842, 864[a]	778-799[b]
	Universal primers (bacteria)		
636	5'-ACT GGY GTT GAI ATG TTC CGY AA	7,54,78, 100,103,159, 209,224,227[b]	470-492[d]
637[c]	5'-ACG TCA GTI GTA CGG AAR TAG AA	7,54,78, 100,103,159, 209,224,227[b]	692-714[d]
638	5'-CCA ATG CCA CAA ACI CGT GAR CAC AT	7,54,78, 100,103,159, 209,224,227[b]	35-60[e]
639[c]	5'-TTT ACG GAA CAT TTC WAC ACC WGT IAC A	7,54,78, 100,103,159, 209,224,227[b]	469-496[e]

a These sequences were aligned to derive the corresponding primer.

b The nucleotide positions refer to the *L. tropica tuf* (EF-1) sequence fragment (SEQ ID NO. 526).

c These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

d The nucleotide positions refer to the *E. coli tuf* sequence fragment (SEQ ID NO. 78).

e The nucleotide positions refer to the *B. cereus tuf* sequence fragment (SEQ ID NO. 7).

Annex I: Specific and ubiquitous prim rs for nucleic acid amplification (*tuf* sequences) (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment	
		SEQ ID NO.	Nucleotide position
	Universal primers (bacteria) (continued)		
643	5'-ACT GGI GTI GAR ATG TTC CGY AA	1,3,4,7,12, 13,16,49,54, 72,78,85,88, 91,94,98,103, 108,112,115, 116,120,121, 126,128,134, 136,146,154, 159,179,186, 205,209,212, 224,238[a]	470-492[b]
644[c]	5'-ACG TCI GTI GTI CKG AAR TAG AA	same as SEQ ID NO. 643	692-714[b]
643	5'-ACT GGI GTI GAR ATG TTC CGY AA	1,3,4,7,12, 13,16,49,54, 72,78,85,88, 91,94,98,103, 108,112,115, 116,120,121, 126,128,134, 136,146,154, 159,179,186, 205,209,212, 224,238[a]	470-492[b]
645[c]	5'-ACG TCI GTI GTI CKG AAR TAR AA	same as SEQ ID NO. 643	692-714[b]
646	5'-ATC GAC AAG CCI TTC YTI ATG SC	2,13,82 122,145[a]	317-339[d]
647[c]	5'-ACG TCC GTS GTR CGG AAG TAG AAC TG	2,13,82 122,145[a]	686-711[d]
646	5'-ATC GAC AAG CCI TTC YTI ATG SC	2,13,82 122,145[a]	317-339[d]
648[c]	5'-ACG TCS GTS GTR CGG AAG TAG AAC TG	2,13,82 122,145[a]	686-711[d]

[a] These sequences were aligned to derive the corresponding primer.

[b] The nucleotide positions refer to the *E. coli tuf* sequence fragment (SEQ ID NO. 78).

[c] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

[d] The nucleotide positions refer to the *A. meyeri tuf* sequence fragment (SEQ ID NO. 2)

Annex I: Specific and ubiquitous prim rs for nucleic acid amplification (*tuf* sequences) (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment	
		SEQ ID NO.	Nucleotide position
	Universal primers (bacteria) (continued)		
649	5'-GTC CTA TGC CTC ARA CWC GIG AGC AC	8,86,141,143[a]	33-58[b]
650[c]	5'-TTA CGG AAC ATY TCA ACA CCI GT	8,86,141,143[a]	473-495[b]
636	5'-ACT GGY GTT GAI ATG TTC CGY AA	8,86,141,143[a]	473-495[b]
651[c]	5'-TGA CGA CCA CCI TCY TCY TTY TTC A	8,86,141,143[a]	639-663[b]
	Universal primers (fungi)		
1974	5'-ACA AGG GIT GGR MSA AGG AGA C	404,405,433, 445,898,1268, 1276,1986[a]	443-464[d]
1975[c]	5'-TGR CCR GGG TGG TTR AGG ACG	404,405,433, 445,898,1268, 1276,1986[a]	846-866[d]
1976	5'-GAT GGA YTC YGT YAA ITG GGA	407-412, 414-426,428-431,439,443,447, 448,622,624,665, 1685,1987-1990[a]	286-306[e]
1978[c]	5'-CAT CIT GYA ATG GYA ATC TYA AT	same as SEQ ID NO. 1976	553-575[e]
1977	5'-GAT GGA YTC YGT YAA RTG GGA	same as SEQ ID NO. 1976	286-306[e]
1979[c]	5'-CAT CYT GYA ATG GYA ASC TYA AT	same as SEQ ID NO. 1976	553-575[e]
1981	5'-TGG ACA CCI SCA AGI GGK CYG	401-405, 433,435,436, 438,444,445,449, 453,455,457,779, 781-783,785,786, 788-790,897-903, 1267-1272,1274-1280, 1282-1287,1991-1998[a]	281-301[d]
1980[c]	5'-TCR ATG GCI TCI AIR AGR GTY T	same as SEQ ID NO. 1981	488-509[d]

[a] These sequences were aligned to derive the corresponding primer.
[b] The nucleotide positions refer to the *B. distasonis tuf* sequence fragment (SEQ ID NO. 8).
[c] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.
[d] The nucleotide positions refer to the *A. fumigatus tuf* (EF-1) sequence fragment (SEQ ID NO. 404).
[e] The nucleotide positions refer to the *C. albicans tuf* (EF-1) sequence fragment (SEQ ID NO. 407).

Annex I: Specific and ubiquitous primers for nucleic acid amplification (tuf sequences) (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment	
		SEQ ID NO.	Nucleotide position
	Universal primers (fungi) (continued)		
1982	5'-TGG ACA CYI SCA AGI GGK CYG	same as SEQ ID NO. 1981	281-301[a]
1980[b]	5'-TCR ATG GCI TCI AIR AGR GTY T	same as SEQ ID NO. 1981	488-509[a]
1983	5'-CYG AYT GCG CYA TIC TCA TCA	same as SEQ ID NO. 1981	143-163[a]
1980[b]	5'-TCR ATG GCI TCI AIR AGR GTY T	same as SEQ ID NO. 1981	488-509[a]
1984	5'-CYG AYT GYG CYA TYC TSA TCA	same as SEQ ID NO. 1981	143-163[a]
1980[b]	5'-TCR ATG GCI TCI AIR AGR GTY T	same as SEQ ID NO. 1981	488-509[a]
	Sequencing primers		
556	5'-CGG CGC NAT CYT SGT TGT TGC	668[c]	306-326
557[b]	5'-CCM AGG CAT RAC CAT CTC GGT G	668[c]	1047-1068
694	5'-CGG CGC IAT CYT SGT TGT TGC	668[c]	306-326
557b	5'-CCM AGG CAT RAC CAT CTC GGT G	668[c]	1047-1068
664	5'-AAY ATG ATI ACI GGI GCI GCI CAR ATG GA	619[c]	604-632
652[b]	5'-CCW AYA GTI YKI CCI CCY TCY CTI ATA	619[c]	1482-1508
664	5'-AAY ATG ATI ACI GGI GCI GCI CAR ATG GA	619[c]	604-632
561[b]	5'-ACI GTI CGG CCR CCC TCA CGG AT	619[c]	1483-1505
543	5'-ATC TTA GTA GTT TCT GCT GCT GA	607	8-30
660[b]	5'-GTA GAA TTG AGG ACG GTA GTT AG	607	678-700
658	5'-GAT YTA GTC GAT GAT GAA GAA TT	621	116-138
659[b]	5'-GCT TTT TGI GTT TCW GGT TTR AT	621	443-465
658	5'-GAT YTA GTC GAT GAT GAA GAA TT	621	116-138
661[b]	5'-GTA GAA YTG TGG WCG ATA RTT RT	621	678-700
558	5'-TCI TTY AAR TAY GCI TGG GT	665[c]	157-176
559[b]	5'-CCG ACR GCR AYI GTY TGI CKC AT	665[c]	1279-1301
813	5'-AAT CYG TYG AAA TGC AYC ACG A	665[c]	687-708
559[b]	5'-CCG ACR GCR AYI GTY TGI CKC AT	665[c]	1279-1301

[a] The nucleotide positions refer to the *A. fumigatus tuf* (EF-1) sequence fragment (SEQ ID NO. 404).

[b] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

[c] Sequences from databases.

Annex I: Specific and ubiquitous primers for nucleic acid amplification (*tuf* sequenc s) (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Nucleotide position
	Sequencing primers (continued)		
558	5'-TCI TTY AAR TAY GCI TGG GT	665[a]	157-176
815[b]	5'-TGG TGC ATY TCK ACR GAC TT	665[a]	686-705
560	5'-GAY TTC ATY AAR AAY ATG ATY AC	665[a]	289-311
559[b]	5'-CCG ACR GCR AYI GTY TGI CKC AT	665[a]	1279-1301
653	5'-GAY TTC ATI AAR AAY ATG AT	665[a]	289-308
559[b]	5'-CCG ACR GCR AYI GTY TGI CKC AT	665[a]	1279-1301
558	5'-TCI TTY AAR TAY GCI TGG GT	665[a]	157-176
655[b]	5'-CCR ATA CCI CMR ATY TTG TA	665[a]	754-773
654	5'-TAC AAR ATY KGI GGT ATY GG	665[a]	754-773
559[b]	5'-CCG ACR GCR AYI GTY TGI CKC AT	665[a]	1279-1301
696	5'-ATI GGI CAY RTI GAY CAY GGI AAR AC	698[a]	52-77
697[b]	5'-CCI ACI GTI CKI CCR CCY TCR CG	698[a]	1132-1154
911	5'-GAC GGM KKC ATG CCG CAR AC	853	22-41
914[b]	5'-GAA RAG CTG CGG RCG RTA GTG	853	700-720
912	5'-GAC GGC GKC ATG CCG CAR AC	846	20-39
914[b]	5'-GAA RAG CTG CGG RCG RTA GTG	846	692-712
913	5'-GAC GGY SYC ATG CCK CAG AC	843	251-270
915[b]	5'-AAA CGC CTG AGG RCG GTA GTT	843	905-925
916	5'-GCC GAG CTG GCC GGC TTC AG	846	422-441
561[b]	5'-ACI GTI CGG CCR CCC TCA CGG AT	619[a]	1483-1505
664	5'-AAY ATG ATI ACI GGI GCI GCI CAR ATG GA	619[a]	604-632
917[b]	5'-TCG TGC TAC CCG TYG CCG CCA T	846	593-614

[a] Sequences from databases.

[b] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex I: Specific and ubiquitous primers for nucleic acid amplification (*tuf* s quences) (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment: SEQ ID NO.	Originating DNA fragment: Nucleotide position
	Sequencing primers (continued)		
1221	5'-GAY ACI CCI GGI CAY GTI GAY TT	1230[a]	292-314
1226[b]	5'-GTI RMR TAI CCR AAC ATY TC	1230[a]	2014-2033
1222	5'-ATY GAY ACI CCI GGI CAY GTI GAY TT	1230[a]	289-314
1223[b]	5'-AYI TCI ARR TGI ARY TCR CCC ATI CC	1230[a]	1408-1433
1224	5'-CCI GYI HTI YTI GAR CCI ATI ATG	1230[a]	1858-1881
1225[b]	5'-TAI CCR AAC ATY TCI SMI ARI GGI AC	1230[a]	2002-2027
1227	5'-GTI CCI YTI KCI GAR ATG TTY GGI TA	1230[a]	2002-2027
1229[b]	5'-TCC ATY TGI GCI GCI CCI GTI ATC AT	698[a]	4-29
1228	5'-GTI CCI YTI KCI GAR ATG TTY GGI TAY GC	1230[a]	2002-2030
1229[b]	5'-TCC ATY TGI GCI GCI CCI GTI ATC AT	698[a]	4-29
1999	5'-CAT GTC AAY ATT GGT ACT ATT GGT CAT GT	498-500, 502,505,506, 508,619,2004,2005[c]	25-53[d]
2000[b]	5'-CCA CCY TCI CTC AMG TTG AAR CGT T	same as SEQ ID NO. 1999	1133-1157[d]
2001	5'-ACY ACI TTR ACI GCY GCY ATY AC	same as SEQ ID NO. 1999	67-89[d]
2003[b]	5'-CAT YTC RAI RTT GTC ACC TGG	same as SEQ ID NO. 1999	1072-1092[d]
2002	5'-CCI GAR GAR AGA GCI MGW GGT	same as SEQ ID NO. 1999	151-171[d]
2003[b]	5'-CAT YTC RAI RTT GTC ACC TGG	same as SEQ ID NO. 1999	1072-1092[d]

[a] Sequences from databases.

[b] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

[c] These sequences were aligned to derive the corresponding primer.

[d] The nucleotide positions refer to the *C. albicans tuf* sequence fragment (SEQ ID NO. 2004).

Annex II: Specific and ubiquitous primers for nucleic acid amplification (*atpD* sequ nc s).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment: SEQ ID NO.	Originating DNA fragment: Nucleotide position
Bacterial species:	***Acinetobacter baumannii***		
1690	5'-CAG GTC CTG TTG CGA CTG AAG AA	243	186-208
1691[b]	5'-CAC AGA TAA ACC TGA GTG TGC TTT C	243	394-418
Bacterial species:	***Bacteroides fragilis***		
2134	5'-CGC GTG AAG CTT CTG TG	929	184-200
2135[b]	5'-TCT CGC CGT TAT TCA GTT TC	929	395-414
Bacterial species:	***Bordetella pertussis***		
2180	5'-TTC GCC GGC GTG GGC	1672[c]	544-558
2181[b]	5'-AGC GCC ACG CGC AGG	1672[c]	666-680
Bacterial species:	***Enterococcus faecium***		
1698	5'-GGA ATC AAC AGA TGG TTT ACA AA	292	131-153
1699[b]	5'-GCA TCT TCT GGG AAA GGT GT	292	258-277
1700	5'-AAG ATG CGG AAA GAA GCG AA	292	271-290
1701[b]	5'-ATT ATG GAT CAG TTC TTG GAT CA	292	439-461
Bacterial species:	***Klebsiella pneumoniae***		
1331	5'-GCC CTT GAG GTA CAG AAT GGT AAT GAA GTT	317	88-118
1332[b]	5'-GAC CGC GGC GCA GAC CAT CA	317	183-203

[a] These sequences were aligned to derive the corresponding primer.

[b] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

[c] Sequence from databases.

Annex II: Specific and ubiquitous prim rs for nucleic acid amplification (*atpD* sequences).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment: SEQ ID NO.	Originating DNA fragment: Nucleotide position
Bacterial species:	***Streptococcus agalactiae***		
627	5'-ATT GTC TAT AAA AAT GGC GAT AAG TC	379-383[a]	42-67[b]
625[c]	5'-CGT TGA AGA CAC GAC CCA AAG TAT CC	379-383[a]	206-231[b]
628	5'-AAA ATG GCG ATA AGT CAC AAA AAG TA	379-383[a]	52-77[b]
625[c]	5'-CGT TGA AGA CAC GAC CCA AAG TAT CC	379-383[a]	206-231[b]
627	5'-ATT GTC TAT AAA AAT GGC GAT AAG TC	379-383[a]	42-67[b]
626[c]	5'-TAC CAC CTT TTA AGT AAG GTG CTA AT	379-383[a]	371-396[b]
628	5'-AAA ATG GCG ATA AGT CAC AAA AAG TA	379-383[a]	52-77[b]
626[c]	5'-TAC CAC CTT TTA AGT AAG GTG CTA AT	379-383[a]	371-396[b]
Bacterial group:	***Campylobacter jejuni* and *C. coli***		
2131	5'-AAG CMA TTG TTG TAA ATT TTG AAA G	1576,1600, 1849,1863,2139[d,a]	7-31[e]
2132[c]	5'-TCA TAT CCA TAG CAA TAG TTC TA	1576,1600, 1849,1863,2139[d,a]	92-114[e]
Bacterial genus:	***Bordetella* sp.**		
825	5'-ATG AGC ARC GSA ACC ATC GTT CAG TG	1672[d]	1-26
826[c]	5'-TCG ATC GTG CCG ACC ATG TAG AAC GC	1672[d]	1342-1367
Fungal genus:	***Candida* sp.**		
634	5'-AAC ACY GTC AGR RCI ATT GCY ATG GA	460-472, 474-478[a]	101-126[f]
635[c]	5'-AAA CCR GTI ARR GCR ACT CTI GCT CT	460-472, 474-478[a]	617-642[f]

a These sequences were aligned to derive the corresponding primer.
b The nucleotide positions refer to the *S. agalactiae atpD* sequence fragment (SEQ ID NO. 380).
c These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.
d Sequence from databases.
e The nucleotide positions refer to the *C. jejuni atpD* sequence fragment (SEQ ID NO. 1576).
f The nucleotide positions refer to the *C. albicans atpD* sequence fragment (SEQ ID NO. 460).

Annex II: Specific and ubiquitous primers for nucleic acid amplification (*atpD* sequences) (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment	
		SEQ ID NO.	Nucleotide position
	Universal primers		
562	5'-CAR ATG RAY GAR CCI CCI GGI GYI MGI ATG	243,244,262, 264,280,284, 291,297,309, 311,315,317, 324,329,332, 334-336,339, 342,343,351, 356,357,364-366,370,375, 379,393[a]	528-557[b]
563[c]	5'-GGY TGR TAI CCI ACI GCI GAI GGC AT	243,244,262, 264,280,284, 291,297,309, 311,315,317, 324,329,332, 334-336,339, 342,343,351, 356,357,364-366,370,375, 379,393[a]	687-712[b]
564	5'-TAY GGI CAR ATG AAY GAR CCI CCI GGI AA	243,244,262, 264,280,284, 291,297,309, 311,315,317, 324,329,332, 334-336,339, 342,343,351, 356,357,364-366,370,375, 379,393[a]	522-550[b]
565[c]	5'-GGY TGR TAI CCI ACI GCI GAI GGD AT	243,244,262, 264,280,284, 291,297,309, 311,315,317, 324,329,332, 334-336,339, 342,343,351, 356,357,364-366,370,375, 379,393[a]	687-712[b]

[a] These sequences were aligned to derive the corresponding primer.

[b] The nucleotide positions refer to the *K. pneumoniae atpD* sequence fragment (SEQ ID NO. 317).

[c] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex II: Specific and ubiquitous primers for nucleic acid amplification (*atpD* sequences) (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
	Universal primers (continued)		
640	5'-TCC ATG GTI TWY GGI CAR ATG AA	248,284,315, 317,343,357, 366,370,379,393[a]	513-535[b]
641[c]	5'-TGA TAA CCW ACI GCI GAI GGC ATA CG	248,284,315, 317,343,357, 366,370,379,393[a]	684-709[b]
642	5'-GGC GTI GGI GAR CGI ACI CGT GA	248,284,315, 317,343,357, 366,370,379,393[a]	438-460[b]
641[c]	5'-TGA TAA CCW ACI GCI GAI GGC ATA CG	248,284,315, 317,343,357, 366,370,379,393[a]	684-709[b]
	Sequencing primers		
566	5'-TTY GGI GGI GCI GGI GTI GGI AAR AC	669[d]	445-470
567[c]	5'-TCR TCI GCI GGI ACR TAI AYI GCY TG	669[d]	883-908
566	5'-TTY GGI GGI GCI GGI GTI GGI AAR AC	669[d]	445-470
814	5'-GCI GGC ACG TAC ACI GCC TG	666[d]	901-920
568	5'-RTI ATI GGI GCI GTI RTI GAY GT	669[d]	25-47
567[c]	5'-TCR TCI GCI GGI ACR TAI AYI GCY TG	669[d]	883-908
570	5'-RTI RYI GGI CCI GTI RTI GAY GT	672[d]	31-53
567[c]	5'-TCR TCI GCI GGI ACR TAI AYI GCY TG	669[d]	883-908
572	5'-RTI RTI GGI SCI GTI RTI GA	669[d]	25-44
567[c]	5'-TCR TCI GCI GGI ACR TAI AYI GCY TG	669[d]	883-908
569	5'-RTI RTI GGI SCI GTI RTI GAT AT	671[d]	31-53
567[c]	5'-TCR TCI GCI GGI ACR TAI AYI GCY TG	669[d]	883-908
571	5'-RTI RTI GGI CCI GTI RTI GAT GT	670[d]	31-53
567[c]	5'-TCR TCI GCI GGI ACR TAI AYI GCY TG	669[d]	883-908

[a] These sequences were aligned to derive the corresponding primer.

[b] The nucleotide positions refer to the *K. pneumoniae atpD* sequence fragment (SEQ ID NO. 317).

[c] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

[d] Sequences from databases.

Annex II: Specific and ubiquitous primers for nucleic acid amplification (*atpD* sequences) (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment: SEQ ID NO.	Originating DNA fragment: Nucleotide position
	Sequencing primers (continued)		
700	5'-TIR TIG AYG TCG ART TCC CTC ARG	669[a]	38-61
567[b]	5'-TCR TCI GCI GGI ACR TAI AYI GCY TG	669[a]	883-908
568	5'-RTI ATI GGI GCI GTI RTI GAY GT	669[a]	25-47
573[b]	5'-CCI CCI ACC ATR TAR AAI GC	666[a]	1465-1484
574	5'-ATI GCI ATG GAY GGI ACI GAR GG	666[a]	283-305
573[b]	5'-CCI CCI ACC ATR TAR AAI GC	666[a]	1465-1484
574	5'-ATI GCI ATG GAY GGI ACI GAR GG	666[a]	283-305
708[b]	5'-TCR TCC ATI CCI ARI ATI GCI ATI AT	666[a]	1258-1283
681	5'-GGI SSI TTY GGI ISI GGI AAR AC	685	694-716
682[b]	5'-GTI ACI GGY TCY TCR AAR TTI CCI CC	686	1177-1202
681	5'-GGI SSI TTY GGI ISI GGI AAR AC	685	694-716
683[b]	5'-GTI ACI GGI TCI SWI AWR TCI CCI CC	685	1180-1205
681	5'-GGI SSI TTY GGI ISI GGI AAR AC	685	694-716
699	5'-GTI ACI GGY TCY TYR ARR TTI CCI CC	686	1177-1202
681	5'-GGI SSI TTY GGI ISI GGI AAR AC	685	694-716
812[b]	5'-GTI ACI GGI TCY TYR ARR TTI CCI CC	685	1180-1205
1213	5'-AAR GGI GGI ACI GCI GCI ATH CCI GG	714[a]	697-722
1212[b]	5'-CCI CCI RGI GGI GAI ACI GCW CC	714[a]	1189-1211
1203	5'-GGI GAR MGI GGI AAY GAR ATG	709[a]	724-744
1207[b]	5'-CCI TCI TCW CCI GGC ATY TC	709[a]	985-1004
1204	5'-GCI AAY AAC ITC IWM YAT GCC	709[a]	822-842
1206[b]	5'-CKI SRI GTI GAR TCI GCC A	709[a]	926-944
1205	5'-AAY ACI TCI AWY ATG CCI GT	709[a]	826-845
1207[b]	5'-CCI TCI TCW CCI GGC ATY TC	709[a]	985-1004
2282	5'-AGR RGC IMA RAT GTA TGA	714[a]	84-101
2284[b]	5'-TCT GWG TRA CIG GYT CKG AGA	714[a]	1217-1237
2283	5'-ATI TAT GAY GGK ITT CAG AGG C	714[a]	271-292
2285[b]	5'-CMC CIC CWG GTG GWG AWA C	714[a]	1195-1213

[a] Sequences from databases.

[b] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex III: Internal hybridization probes for specific detection of *tuf* sequences.

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Bacterial species:	***Abiotrophia adiacens***		
2170	5'-ACG TGA CGT TGA CAA ACC A	1715	313-331
Bacterial species:	***Chlamydia pneumoniae***		
2089	5'-ATG CTG AAC TTA TTG ACC TT	20	136-155
2090	5'-CGT TAC TGG AGT CGA AAT G	20	467-485
Bacterial species:	***Enterococcus faecalis***		
580	5'-GCT AAA CCA GCT ACA ATC ACT CCA C	62-63,607[a]	584-608[b]
603	5'-GGT ATT AAA GAC GAA ACA TC	62-63,607[a]	440-459[b]
1174	5'-GAA CGT GGT GAA GTT CGC	62-63,607[a]	398-415[b]
Bacterial species:	***Enterococcus faecium***		
602	5'-AAG TTG AAG TTG TTG GTA TT	64,608[a]	426-445[c]
Bacterial species:	***Enterococcus gallinarum***		
604	5'-GGT GAT GAA GTA GAA ATC GT	66,609[a]	419-438[d]
Bacterial species:	***Escherichia coli***		
579	5'-GAA GGC CGT GCT GGT GAG AA	78	503-522
2168	5'-CAT CAA AGT TGG TGA AGA AGT TG	78	409-431
Bacterial species:	***Neisseria gonorrhoeae***		
2166	5'-GAC AAA CCA TTC CTG CTG	126	322-339[e]
Fungal species:	***Candida albicans***		
577	5'-CAT GAT TGA ACC ATC CAC CA	407-411[a]	406-425[f]
Fungal species:	***Candida dubliniensis***		
578	5'-CAT GAT TGA AGC TTC CAC CA	412,414-415[a]	418-437[g]

[a] These sequences were aligned to derive the corresponding primer.
[b] The nucleotide positions refer to the *E. faecalis tuf* sequence fragment (SEQ ID NO. 607).
[c] The nucleotide positions refer to the *E. faecium tuf* sequence fragment (SEQ ID NO. 608).
[d] The nucleotide positions refer to the *E. gallinarum tuf* sequence fragment (SEQ ID NO. 609).
[e] The nucleotide positions refer to the *N. gonorrhoeae tuf* sequence fragment (SEQ ID NO. 126).
[f] The nucleotide positions refer to the *C. albicans tuf*(EF-1) sequence fragment (SEQ ID NO. 408).
[g] The nucleotide positions refer to the *C. dubliniensis tuf*(EF-1) sequence fragment (SEQ ID NO. 414).

Annex III: Internal hybridization probes for specific detection of *tuf* sequences (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment: SEQ ID NO.	Originating DNA fragment: Nucleotide position
Bacterial species:	***Haemophilus influenzae***		
581	5'-ACA TCG GTG CAT TAT TAC GTG G	610[a]	551-572
Bacterial species:	***Mycoplasma pneumoniae***		
2095	5'-CGG TCG GGT TGA ACG TGG	2097[a]	687-704
Bacterial species:	***Staphylococcus aureus***		
584	5'-ACA TGA CAC ATC TAA AAC AA	176-180[b]	369-388[c]
585	5'-ACC ACA TAC TGA ATT CAA AG	176-180[b]	525-544[c]
586	5'-CAG AAG TAT ACG TAT TAT CA	176-180[b]	545-564[c]
587	5'-CGT ATT ATC AAA AGA CGA AG	176-180[b]	555-574[c]
588	5'-TCT TCT CAA ACT ATC GTC CA	176-180[b]	593-612[c]
Bacterial species:	***Staphylococcus epidermidis***		
589	5'-GCA CGA AAC TTC TAA AAC AA	185,611[b]	445-464[d]
590	5'-TAT ACG TAT TAT CTA AAG AT	185,611[b]	627-646[d]
591	5'-TCC TGG TTC TAT TAC ACC AC	185,611[b]	586-605[d]
592	5'-CAA AGC TGA AGT ATA CGT AT	185,611[b]	616-635[d]
593	5'-TTC ACT AAC TAT CGC CCA CA	185,611[b]	671-690[d]
Bacterial species:	***Staphylococcus haemolyticus***		
594	5'-ATT GGT ATC CAT GAC ACT TC	186,188-190[b]	437-456[e]
595	5'-TTA AAG CAG ACG TAT ACG TT	186,188-190[b]	615-634[e]
Bacterial species:	***Staphylococcus hominis***		
596	5'-GAA ATT ATT GGT ATC AAA GA	191,193-196[b]	431-450[f]
597	5'-ATT GGT ATC AAA GAA ACT TC	191,193-196[b]	437-456[f]
598	5'-AAT TAC ACC TCA CAC AAA AT	191,193-196[b]	595-614[f]

[a] Sequences from databases.

[b] These sequences were aligned to derive the corresponding probe.

[c] The nucleotide positions refer to the *S. aureus tuf* sequence fragment (SEQ ID NO. 179).

[d] The nucleotide positions refer to the *S. epidermidis tuf* sequence fragment (SEQ ID NO. 611).

[e] The nucleotide positions refer to the *S. haemolyticus tuf* sequence fragment (SEQ ID NO. 186).

[f] The nucleotide positions refer to the *S. hominis tuf* sequence fragment (SEQ ID NO. 191).

Annex III: Internal hybridization probes for specific detection of *tuf* sequences (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Bacterial species:	***Staphylococcus saprophyticus***		
599	5'-CGG TGA AGA AAT CGA AAT CA	198-200[a]	406-425[b]
600	5'-ATG CAA GAA GAA TCA AGC AA	198-200[a]	431-450[b]
601	5'-GTT TCA CGT GAT GAT GTA CA	198-200[a]	536-555[b]
695	5'-GTT TCA CGT GAT GAC GTA CA	198-200[a]	563-582[b]
Bacterial species:	***Streptococcus agalactiae***		
582[c]	5'-TTT CAA CTT CGT CGT TGA CAC GAA CAG T	207-210[a]	404-431[d]
583[c]	5'-CAA CTG CTT TTT GGA TAT CTT CTT TAA TAC CAA CG	207-210[a]	433-467[d]
1199	5'-GTA TTA AAG AAG ATA TCC AAA AAG C	207-210[a]	438-462[d]
Bacterial species:	***Streptococcus pneumoniae***		
1201	5'-TCA AAG AAG AAA CTA AAA AAG CTG T	971,977, 979,986[a]	513-537[e]
Bacterial species:	***Streptococcus pyogenes***		
1200	5'-TCA AAG AAG AAA CTA AAA AAG CTG T	1002	473-497
Bacterial group:	***Enterococcus casseliflavus-flavescens-gallinarum group***		
620	5'-ATT GGT GCA TTG CTA CGT	58,65,66[a]	527-544[f]
1122	5'-TGG TGC ATT GCT ACG TGG	58,65,66[a]	529-546[f]
Bacterial group:	***Enterococcus sp., Gemella sp., A. adiacens***		
2172	5'-GTG TTG AAA TGT TCC GTA AA	58-62,67-71, 87-88,607-609, 727,871 1715,1722[a]	477-496[g]

[a] These sequences were aligned to derive the corresponding primer.

[b] The nucleotide positions refer to the *S. saprophyticus tuf* sequence fragment (SEQ ID NO. 198).

[c] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

[d] The nucleotide positions refer to the *S. agalactiae tuf* sequence fragment (SEQ ID NO. 209).

[e] The nucleotide positions refer to the *S. pneumoniae tuf* sequence fragment (SEQ ID NO. 986).

[f] The nucleotide positions refer to the *E. flavescens tuf* sequence fragment (SEQ ID NO. 65).

[g] The nucleotide positions refer to the *E. faecium tuf* sequence fragment (SEQ ID NO. 608).

Annex III: Internal hybridization probes for specific detection of *tuf* sequences (continu d).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Bacterial genus:	***Gemella***		
2171	5'-TCG TTG GAT TAA CTG AAG AA	87,88[a]	430-449[b]
Bacterial genus:	***Staphylococcus* sp.**		
605	5'-GAA ATG TTC CGT AAA TTA TT	176-203[a]	403-422[c]
606	5'-ATT AGA CTA CGC TGA AGC TG	176-203[a]	420-439[c]
1175	5'-GTT ACT GGT GTA GAA ATG TTC	176-203[a]	391-411[c]
1176	5'-TAC TGG TGT AGA AAT GTT C	176-203[a]	393-411[c]
Bacterial genus:	***Streptococcus* sp.**		
1202	5'-GTG TTG AAA TGT TCC GTA AAC A	206-231,971, 977,979,982-986[a]	466-487[d]
Fungal species:	***Candida albicans***		
1156	5'-GTT GAA ATG CAT CAC GAA CAA TT	407-412,624[a]	680-702[e]
Fungal group:	***Candida albicans* and *C. tropicalis***		
1160	5'-CGT TTC TGT TAA AGA AAT TAG AAG	407-412, 429,624[a]	748-771[e]
Fungal species:	***Candida dubliniensis***		
1166	5'-ACG TTA AGA ATG TTT CTG TCA A	414-415[a]	750-771[f]
1168	5'-GAA CAA TTG GTT GAA GGT GT	414-415[a]	707-726[f]
Fungal species:	***Candida glabrata***		
1158	5'-AAG AGG TAA TGT CTG TGG T	417	781-799
1159	5'-TGA AGG TTT GCC AGG TGA	417	718-735
Fungal species:	***Candida krusei***		
1161	5'-TCC AGG TGA TAA CGT TGG	422	720-737

[a] These sequences were aligned to derive the corresponding primer.

[b] The nucleotide positions refer to the *G. haemolysans tuf* sequence fragment (SEQ ID NO. 87).

[c] The nucleotide positions refer to the *S. aureus tuf* sequence fragment (SEQ ID NO. 179).

[d] The nucleotide positions refer to the *S. pneumoniae tuf* sequence fragment (SEQ ID NO. 986).

[e] The nucleotide positions refer to the *C. albicans tuf*(EF-1) sequence fragment (SEQ ID NO. 408).

[f] The nucleotide positions refer to the *C. dubliniensis tuf*(EF-1) sequence fragment (SEQ ID NO. 414).

Annex III: Internal hybridization prob s for specific detection of *tuf* sequences (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment	
		SEQ ID NO.	Nucleotide position
<u>Fungal group</u>:	***Candida lusitaniae* and *C. guillermondii***		
1162	5'-CAA GTC CGT GGA AAT GCA	418,424[a]	682-699[b]
<u>Fungal species</u>:	***Candida parapsilosis***		
1157	5'-AAG AAC GTT TCA GTT AAG GAA AT	426	749-771
<u>Fungal species</u>:	***Candida zeylanoides***		
1165	5'-GGT TTC AAC GTG AAG AAC	432	713-730
<u>Fungal genus</u>:	***Candida* sp.**		
1163	5'-GTT GGT TTC AAC GTT AAG AAC	407-412,414-415,417,418,422,429[a]	728-748[c]
1164	5'-GGT TTC AAC GTC AAG AAC	413,416,420,421,424,425,426,428,431[a]	740-757[b]
1167	5'-GTT GGT TTC AAC GT	406-426, 428-432, 624[a]	728-741[c]

[a] These sequences were aligned to derive the corresponding primer.

[b] The nucleotide positions refer to the *C. lusitaniae* *tuf*(EF-1) sequence fragment (SEQ ID NO. 424).

[c] The nucleotide positions refer to the *C. albicans* *tuf*(EF-1) sequence fragment (SEQ ID NO. 408).

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Annex IV: *Strategy for the selection of amplification/sequencing primers from* atpD *(F-type) sequences.*

	23 49 443 472 881 910	SEQ ID NO.:	Accession #:
B. cepacia	AGTgCAT CGGCGCCGTT ATCGACGTGG...TGTTCG GCGGTGCTGG CGTGGGCAAG ACCG...TCCA GGCCGTGT ACGTCCCTGC GGACGACT	-	X76877
B. pertussis	AGTgCAT CGGCGCCGTG GTGGATATTC...TGTTCG GCGGCGCCGG CGTGGGCAAG ACCG...TCCA GGCCGTGT ACGTGCCTGC CGACGACT	-	Genome project
P. aeruginosa	AAATCAT CGGCGCCGTG ATCGACGTGG...TGTTCG GCGGCGCCGG CGTGGGCAAG ACCG...TCCA GGCCGTAT ACGTTCCCGC GGACGACC	-	Genome project
E. coli	AGGTAAT CGGCGCCGTA GTTGACGTCG...TGTTCG GTGGTGCGGG TGTAGGTAAA ACCG...TACA GGCAGTAT ACGTACCTGC GGATGACT	-	J01594
N. gonorrhoeae	AAATTAT CGGTGCGGTT GTTGACGTGG...TGTTCG GCGGTGCCGG TGTGGGTAAA ACCG...TCCA AGCCGTAT ATGTACCTGC GGATGACT	-	Genome project
M. thermoacetica	AGGTTAT TGGCCCGGTG GTTGACGTCG...TCTTCG GCGGCGCCGG GGTCGGCAAG ACGG...TGCA AGCTATCT ATGTGCCGGC CGACGACC	-	U64318
S. aurantiaca	AGGTTcT CGGTCCCGTG ATTGACGTGG...TGTTCG GCGGCGCCGG CGTGGGCAAG ACGG...TGCA GGCCATCT ACGTGCCCGC CGACGACC	-	X76879
M. tuberculosis	GGGTCAC TGGGCCCGTC GTCGACGTCG...TGTTCG GCGGTGCCGG GGTGGGCAAG ACGG...TGCA AGCCGTCT ACGTGCCCGC CGACGACT	-	Z73419
B. fragilis	AGGTAAT TGGCCCTGTG GTCGATGTGT...TGTTTG GCGGGGCCGG AGTGGGTAAA ACTG...TGCA GGCTGTTT ACGTACCGGC TGATGACT	-	M22247
C. lytica	AAATTAT TGGCCCAGTT ATAGATGTGG...TATTTG GAGGTGCCGG AGTAGGTAAA ACAG...TACA GGCGGTTT ACGTACCTGC GGATGATT	672	M22535
A. woodii	AGGTTAT TGGACCAGTA GTCGATGTTA...TTTTCG GTGGTGCCGG AGTTGGTAAA ACCG...TTCA GGCCGTTT ACGaTCCAGC CGATGACT	-	U10505
C. acetobutylicum	AGGTAAT AGGACCTGTT GTGGATATTA...TGTTCG GTGGTGCCGG TGTTGGTAAA ACAG...TTCA GGCTGTAT ATGTTCCTGC TGATGACC	671	AF101055
M. pneumoniae	AAGTGAT TGGCCCGGTA GTTGATGTCA...TATTTG GTGGTGCTGG TGTTGGTAAA ACGG...TGCA AGCGATCT ATGTGCCAGC TGATGACT	-	U43738
H. pylori	AGGTTtT AGGCCCGGTG GTAGATGTGG...TGTTTG GTGGGGCTGG CGTAGGCAAA ACGG...TTCA AGCGGTGT ATGTGCCAGC AGACGACT	670	AF004014
Selected sequences for universal primers	RTIAT IGGIGCIGTI RTIGAYGT	568	
	RTIRY IGGICCIGTI RTIGAYGT	570	
	RTIRT IGGISCIGTI RTIGA	572	
	RTIRT IGGISCIGTI RTIGATAT	569	
	RTIRT IGGICCIGTI RTIGATGT	571	
	TTYG GIGGIGCIGG IGTIGGIAAR AC	566	
Selected sequence for universal primer*	CA RGCIRTIT AYGTICCIGC IGAYGA	567	

The sequence numbering refers to the *Escherichia coli atpD* gene fragment (SEQ ID NO. 669). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. Dots indicate gaps in the sequences displayed.

"R" "Y" "M" "K" "W" and "S" designate nucleotide positions which are degenerated. "R" stands for A or G; "Y" stands for C or T; "M" stands for A or C; "K" stands for G or T; "W" stands for A or T; "S" stands for C or G. "I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.

- * This sequence is the reverse-complement of the selected primer.

Annex V: Strategy for the selection of universal amplification/sequencing primers from *atpD* (V-type) sequences.

		SEQ ID NO.:
	691 ... 719 1177 ... 1208	
E. hirae	CC **AGGTCCGTTT GGTGCAGGGA AGACAGT**...TCT**GGTGGAg ATaTCtctGA ACCAGTGACT** CA	685
H. salinarum	CC **GGGGCCGTTC GGGTCCGGGA AGACGGT**...CCC**GGCGGGg ACTTCtccGA GCCGGTCACC** CA	687
T. thermophilus	CC **TGGGCCCTTC GGCAGCGGCA AGACCGT**...CCG**GGCGGCg ACaTgtccGA GCCCGTGACC** CA	693
Human	CC **TGGGGCCTTC GGATGTGGCA AGACTGT**...CCC**GGTGGAg ACTTCtcAGA tCCCGTGACG** AC	688
T. congolense	CC **TGGCGCGTTT GGATGCGGAA AGACGGT**...CCT**GGAGGTg ACTTTtctGA cCCAGTGACG** TC	692
P. falciparum	CC **TGGTGCATTT GGTTGTGGAA AAACTTG**...CCA**GGTGGTg ATTTCtctGA cCCTGTAACT** AC	689
C. pneumoniae	CC **AGGACCTTTT GGTGCAGGGA AAACAGT**...GCA**GGAGGAA ACTTTGAAGA ACCAGTCACT** CA	686
Selected sequences for universal primers	**GGISSITTY GGIISIGGIA ARAC**	681
Selected sequences for universal primers[a]	**GGIGGIA AYTTYGARGA RCCIGTIAC**	682
	GGIGGIG AYWTIWSIGA ICCIGTIAC	683

The sequence numbering refers to the *Enterococcus hirae atpD* gene fragment (SEQ ID NO. 685). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches for SEQ ID NOs. 681 and 682 are indicated by lower-case letters. Mismatches for SEQ ID NO. 683 are indicated by underlined nucleotides. Dots indicate gaps in the sequences displayed.

"R" "Y" "M" "K" "W" and "S" designate nucleotide positions which are degenerated. "R" stands for A or G; "Y" stands for C or T; "M" stands for A or C; "K" stands for G or T; "W" stands for A or T; "S" stands for C or G. "I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.

[a] These sequences are the reverse-complement of the selected primers.

Annex VI: *Strategy for the selection of universal amplification/sequencing primers from* tuf *(M) sequences (organelle origin).*

	601 … 635 1479 … 1511	SEQ ID NO.:	Accession #:
C. neoformans[a]	AAGAA CATGATCACC GGTaCCtCCC AGgctGACTG...CGCcgTCcGA GAcatGcGAC AGACcGTTGc CGT	-	U81803
S. cerevisiae[a]	AAGAA CATGATTACT GGTaCTtCTC AAgctGACTG...CGCTgTCAGA GAcatGaGAC AAACTGTcGc TGT	665	X00779
O. volvulus[a]	AAGAA TATGATCACA GGTaCTtCTC AGgctGACTG...TGCTgTGcGt GAtatGaGAC AAACaGTTGc GGT	-	M64333
Human[a]	AAAAA CATGATTACA GGGaCAtCTC AGgctGACTG...TGCTgTTcGt GAtatGaGAC AGACaGTTGc TGT	-	X03558
G. max B1[b]	AAGAA CATGATCACC GGCGCTGCCC AGATGGACGG...TGCTATTAGA GAAGGAGGCA AAACTGTTGG AGC	-	Y15107
G. max B2[b]	AAAAA CATGATCACC GGCGCCGCCC AGATGGACGG...TGCTATTAGA GAAGGAGGCA AAACTGTTGG AGC	-	Y15108
E. coli[c]	AAAAA CATGATCACC GGTGCTGCTC AGATGGACGG...CGCaATCcGt GAAGGCGGCC GTACcGTTGG CGC	78	-
S. aureofaciens[c]	AAGAA CATGATCACC GGTGCCGCCC AGATGGACGG...CGCcATCcGt GAGGGTGGTC GTACcGTgGG CGC	-	AF007125
E. tenella[b]	AAAAA TATGATTACA GGAGCAGCAC AAATGGATGG...TGCTATAAGA GAAGGAGGAA AAACTATAGG AGC	-	AI755521
T. gondii[b]	AAGAA TATGATTACT GGAGCCGCAC AAATGGATGG...TGCTATTAGA GAAGGAGGTC GTACTATAGG AGC	-	Y11431
S. cerevisiae[b]	AAGAA TATGATTACC GGTGCTGCTC AAATGGATGG...CAATATCAGA GAGGGTGGAA GAACTGTTGG TAC	619	K00428
A. thaliana[b]	AAAAA TATGATTACT GGAGCTGCGC AAATGGATGG...TGCctTAAGG GAAGGAGGTA GAACaGTTGG AGC	-	X89227
Selected sequence for universal primer	AA YATGATIACI GGIGCIGCIC ARATGGA	664	
Selected sequences for universal primers	TATIAGR GARGGIGGIM RIACTRTWGG[d]	652	
	ATCCGT GAGGGYGGCC GITCIGT[d]	561	

The sequence numbering refers to the *Saccharomyces cerevisiae tuf* (M) gene (SEQ ID NO. 619). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches for SEQ ID NOs. 652 and 664 are indicated by lower-case letters. Mismatches for SEQ ID NO. 561 are indicated by underlined nucleotides. Dots indicate gaps in the sequences displayed.

"R" "Y" "M" "K" "W" and "S" designate nucleotide positions which are degenerated. "R" stands for A or G; "Y" stands for C or T; "M" stands for A or C; "K" stands for G or T; "W" stands for A or T; "S" stands for C or G. "I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.

[a] This sequence refers to *tuf*(EF-1) gene.
[b] This sequence refers to *tuf* (M) or organelle gene.
[c] This sequence refers to *tuf* gene from bacteria.
[d] These sequences are the reverse-complement of the selected primers.

Annex VII: Strategy for the selection of eukaryotic sequencing primers from tuf (EF-1) sequences.

	154 179 286 314	SEQ ID NO.:	Accession #:
S. cerevisiae	GG **TTCTTTCAAG TACGCTTGGG** TTTT...AGAGA **TTTCATCAAG AACATGATTA** CTGG...	665	X00779
B. hominis	GG **CTCCTTCAAG TACGCGTGGG** TGCT...CGTGA **CTTCATaAAG AACATGATCA** CGGG...	-	D64080
C. albicans	GG **TTCTTTCAAA TACGCTTGGG** TCTT...AGAGA **TTTCATCAAG AATATGATCA** CTGG...	-	M29934
C. neoformans	TC **TTCTTTCAAG TACGCTTGGG** TTCT...CGAGA **CTTCATCAAG AACATGATCA** CCGG...	-	U81803
E. histolytica	GG **ATCATTCAAA TATGCTTGGG** TCTT...AGAGA **TTTCATTAAG AACATGATTA** CTGG...	-	M92073
G. lamblia	GG **CTCCTTCAAG TACGCGTGGG** TCCT...CGCGA **CTTCATCAAG AACATGATCA** CGGG...	-	D14342
H. capsulatum	AA **ATCCTTCAAA TATGCGTGGG** TCCT...CGTGA **CTTCATCAAG AACATGATCA** CTGG...	-	U14100
Human	GG **CTCCTTCAAG TATGCCTGGG** TCTT...AGAGA **CTTtATCAAA AACATGATTA** CAGG...	-	X03558
L. braziliensis	GC **GTCCTTCAAG TACGCGTGGG** TGCT...CGCGA **CTTCATCAAG AACATGATCA** CCGG...	-	U72244
O. volvulus	GG **CTCATTTAAA TATGCTTGGG** TATT...CGTGA **TTTCATTAAG AATATGATCA** CAGG...	-	M64333
P. berghei	GG **TagTTTCAAA TATGCATGGG** TTTT...AAAcA **TTTtATTAAA AATATGATTA** CTGG...	-	AJ224150
P. knowlesi	GG **AagTTTTAAG TACGCATGGG** TGTT...AAGGA **TTTCATTAAA AATATGATTA** CCGG...	-	AJ224153
S. pombe	GG **TTCCTTCAAG TACGCCTGGG** TTTT...CGTGA **TTTCATCAAG AACATGATTA** CCGG...	-	U42189
T. cruzi	TC **TTCTTTCAAG TACGCGTGGG** TCTT...CGCGA **CTTCATCAAG AACATGATCA** CGGG...	-	L76077
Y. lipolytica	GG **TTCTTTCAAG TACGCTTGGG** TTCT...CGAGA **TTTCATCAAG AACATGATCA** CCGG...	-	AF054510
Selected sequences for amplification primers	**TCITTYAAR TAYGCITGGG T**	558	
	GA YTTCATYAAR AAYATGATYA C	560	
	GA YTTCATIAAR AAYATGAT	653	

The sequence numbering refers to the *Saccharomyces cerevisiae tuf* (EF-1) gene fragment (SEQ ID NO. 665). Nucleotides in capitals are identical to the selected sequences SEQ ID NOs. 558, 560 or 653, or match those sequences. Mismatches for SEQ ID no. 558 and 560 are indicated by lower-case letters. Mismatches for SEQ ID NO. 653 are indicated by underlined nucleotides. Dots indicate gaps in the sequences displayed.

"R" "Y" "M" "K" "W" and "S" designate nucleotide positions which are degenerated. "R" stands for A or G; "Y" stands for C or T; "M" stands for A or C; "K" stands for G or T; "W" stands for A or T; "S" stands for C or G. "I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.

Annex VII: Strategy for the selection of eukaryotic sequencing primers from tuf (EF-1) sequences (continued).

	751 ... 776	1276 ... 1304	SEQ ID NO.:	Accession #:
S. cerevisiae	...GTTTACAA GATCGGTGGT ATTGGTAC...	...GACATG AGACAAACTG TCGCTGTCGG TGT	665	X00779
B. hominis	...GTGTACAA GATTGGCGGT ATTGGTAC...	...GATATG AGACAGACTG TCGCTGTCGG TAT	-	D64080
C. albicans	...GTTTACAA GATCGGTGGT ATTGGTAC...	...GATATG AGACAAACCG TTGCTGTtGG TGT	-	M29934
C. neoformans	...GTCTACAA GATCGGTGGT ATCGGCAC...	...GACATG CGACAGACCG TTGCCGTtGG TGT	-	U81803
E. histolytica	...GTTTACAA GATTTcAGGT ATTGGAAC...	...GATATG AaACAAACCG TTGCTGTtGG AGT	-	M92073
G. lamblia	...GTCTACAA GATCTcGGGc gTCGGGAC...	~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~	-	D14342
H. capsulatum	...GTGTACAA AATCTcTGGT ATTGGCAC...	...GACATG AGACAAACCG TCGCTGTCGG TGT	-	U14100
Human	...GTCTACAA AATTGGTGGT ATTGGTAC...	...GATATG AGACAGACAG TTGCgGTgGG TGT	-	X03558
L. braziliensis	...GTGTACAA GATCGGCGGT ATCGGCAC...	...GACATG CGCagAACGG TCGCCGTCGG CAT	-	U72244
O. volvulus	...GTTTACAA AATTGGAGGT ATTGGAAC...	...GATATG AGACAAACAG TTGCTGTtGG CGT	-	M64333
P. berghei	...GTATACAA AATTGGTGGT ATTGGTAC...	...GATATG AGACAAACAA TTGCTGTCGG TAT	-	AJ224150
P. knowlesi	...GTATACAA AATCGGTGGT ATTGGTAC...	...GATATG AGACAAACCA TTGCTGTCGG TAT	-	AJ224153
S. pombe	...GTTTACAA GATCGGTGGT ATTGGTAC...	...GACATG CGTCAAACCG TCGCTGTCGG TGT	-	U42189
T. cruzi	...GTGTACAA GATCGGCGGT ATCGGCAC...	...GACATG CGCCAGACGG TCGCCGTCGG CAT	-	L76077
Y. lipolytica	...GTCTACAA GATCGGTGGT ATCGGCAC...	...GACATG CGACAGACCG TTGCTGTCGG TGT	-	AF054510
Selected sequence for amplification primer	TACAA RATYKGIGGT ATYGG		654	
Selected sequences for amplification primers[a]	TACAA RATYKGIGGT ATYGG		655	
		ATG MGICARACIR TYGCYGTCGG	559	

The sequence numbering refers to the *Saccharomyces cerevisiae tuf* (EF-1) gene fragment (SEQ ID NO. 665). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. "~" indicate incomplete sequence data. Dots indicate gaps in the sequences displayed.

"R" "Y" "M" "K" "W" and "S" designate nucleotide positions which are degenerated. "R" stands for A or G; "Y" stands for C or T; "M" stands for A or C; "K" stands for G or T; "W" stands for A or T; "S" stands for C or G. "I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.

[a] This sequences are the reverse-complement of the selected primers.

Annex VIII: *Strategy for the selection of* Streptococcus agalactiae*-specific amplification primers from tuf sequences.*

	305 … 334 517 … 542	SEQ ID NO.:	Accession #:
S. agalactiae	CCAGAA CGTGATACTG ACAAACCTTT ACTT...GGAC AACGTTGGTG TTCTTCTTCG TG	207	-
S. agalactiae	CCAGAA CGTGATACTG ACAAACCTTT ACTT...GGAC AACGTTGGTG TTCTTCTTCG TG	208	-
S. agalactiae	CCAGAA CGTGATACTG ACAAACCTTT ACTT...GGAC AACGTTGGTG TTCTTCTTCG TG	209	-
S. agalactiae	CCAGAA CGTGATACTG ACAAACCTTT ACTT...GGAC AACGTTGGTG TTCTTCTTCG TG	210	-
S. anginosus	CCAGAA CGTGAcACTG ACAAACCaTT gCTT...AGAt AACGTaGGgG TTCTTCTTCG TG	211	-
S. anginosus	CCAGAA CGTGATACTG ACAAACCaTT gCTT...AGAt AACGTaGGgG TTCTTCTTCG TG	221	-
S. bovis	CCAaAA CGTGATACTG ACAAACCaTT gCTT...GGAt AACGTTGGTG TTCTTCTTCG TG	212	-
S. gordonii	CCAGAA CGTGAcACTG ACAAACCaTT gCTT...AGAt AAtGTaGGTG TcCTTCTTCG TG	223	-
S. mutans	CCAGAA CGTGATACTG ACAAgCCgcT cCTT...GGAt AAtGTTGGTG TTCTcCTTCG TG	224	-
S. pneumoniae	CCAGAA CGTGAcACTG ACAAACCaTT gCTT...AGAt AACGTaGGTG TcCTTCTTCG TG	145[a]	
S. sanguinis	CCAGAA CGcGATACTG ACAAgCCaTT gCTT...GGAC AACGTaGGTG TgCTTCTcCG TG	227	-
S. sobrinus	CCAaAA CGcGATACTG AtAAgCCaTT gCTT...AGAt AACGTTGGTG TgCTTCTTCG TG	228	-
B. cepacia	CCGGAg CGTGcagtTG ACggcgCgTT cCTG...CGAC AACGTTGGTa TcCTgCTgCG cG	16	-
B. fragilis	CCTccg CGcGATgtTG AtAAACCTTT ctTG...TGAC AACGTaGGTc TgtTgCTTCG TG	-	P33165
B. subtilis	CCAGAA CGcGAcACTG AaAAACCaTT caTG...TGAC AACaTTGGTG ccCTTCTTCG cG	-	Z99104
C. diphtheriae	CCAGAg CGTGAgACcG ACAAgCCaTT cCTC...CGAC AACtgTGGTc TgCTTCTcCG TG	662	-
C. trachomatis	CCAGAA aGaGAaAtTG ACAAgCCTTT cTTA...AGAg AAtGTTGGat TgCTcCTcaG aG	22	-
E. coli	CCAGAg CGTGcgAtTG ACAAgCCgTT cCTg...TGAg AACGTaGGTG TTCTgCTgCG TG	78	-
G. vaginalis	CCAact CacGATctTG ACAAgCCaTT cTTg...CGAt RACacTGGTc TTCTTCTcCG cG	135[a]	
S. aureus	CCAGAA CGTGATtCTG ACAAACCaTT cATg...TGAC AACaTTGGTG catTatTaCG TG	179	-
Selected sequence for species-specific primer	GAA CGTGATACTG ACAAACCTTT A	549	
Selected sequence for species-specific primer[b]	C AACGTTGGTG TTCTTCTTC	550	

The sequence numbering refers to the *Streptococcus agalactiae tuf* gene fragment (SEQ ID NO. 209). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. Dots indicate gaps in the sequences displayed.

"R" "Y" "M" "K" "W" and "S" designate nucleotide positions which are degenerated. "R" stands for A or G; "Y" stands for C or T; "M" stands for A or C; "K" stands for G or T; "W" stands for A or T; "S" stands for C or G. "I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.

[a] The SEQ ID NO. refers to previous patent publication WO98/20157.
[b] This sequence is the reverse-complement of the selected primer.

Ann x IX: *Strategy for the selection of* Streptococcus agalactiae-*specific hybridization probes from* tuf *sequences.*

	401			431 433				470	SEQ ID NO.:	Accession #:
S. acidominimus	GGTACTGT	TaaaGTtAAt	GACGAAGTTG	AAATCGTTGG	TATcAAAGAc	GAaATCtctA	AAGCAGTTGT	TA	206	
S. agalactiae	GGTACTGT	TCGTGTCAAC	GACGAAGTTG	AAATCGTTGG	TATTAAAGAA	GATATCCAAA	AAGCAGTTGT	TA	209	
S. agalactiae	GGTACTGT	TCGTGTCAAC	GACGAAGTTG	AAATCGTTGG	TATTAAAGAA	GATATCCAAA	AAGCAGTTGT	TA	144[a]	
S. agalactiae	GGTACTGT	TCGTGTCAAC	GACGAAGTTG	AAATCGTTGG	TATTAAAGAA	GATATCCAAA	AAGCAGTTGT	TA	207	
S. agalactiae	GGTACTGT	TCGTGTCAAC	GACGAAGTTG	AAATCGTTGG	TATTAAAGAA	GATATCCAAA	AAGCAGTTGT	TA	210	
S. agalactiae	GGTACTGT	TCGTGTCAAC	GACGAAGTTG	AAATCGTTGG	TATTAAAGAA	GATATCCAAA	AAGCAGTTGT	TA	208	
S. anginosus	GGTACTGT	TaaaGTCAAC	GACGAAGTTG	AAATCGTTGG	TATccgtGAt	GAaATCCAAA	AAGCAGTTGT	TA	211	
S. anginosus	GGTACTGT	TaaaGTCAAC	GAtGAAGTTG	AAATCGTTGG	TATccgcGAg	GAaATCCAAA	AAGCAGTTGT	TA	221	
S. bovis	GGTACTGT	TaaaGTCAAC	GACGAAGTTG	AAATCGTTGG	TATccgtGAc	GAcATCCAAA	AAGCtGTTGT	TA	212	
S. anginosus	GGTACTGT	TaaaGTCAAt	GAtGAAGTTG	AAATtGTTGG	TATTcgtGAc	GAaATCCAAA	AAGCAGTTGT	TA	213	
S. cricetus	GGTACTGT	TaagGTCAAt	GACGAAGTTG	AAATCGTTGG	TATcAAgGAc	GAaATCCAAA	AAGCgGTTGT	TA	214	
S. cristatus	GGTACTGT	TCGTGTCAAC	GAtGAAaTcG	AAATCGTTGG	TATcAAAGAA	GAaATCCAAA	AAGCAGTTGT	TA	215	
S. downei	GGTACTGT	TaagGTCAAC	GACGAAGTTG	AAATCGTTGG	TATcAAgGAc	GAaATCCAAA	AAGCAGTTGT	TA	216	
S. dysgalactiae	GGTACTGT	TCGTGTCAAC	GACGAAaTcG	AAATCGTTGG	TATcAAAGAA	GAaActaAAA	AAGCtGTTGT	TA	217	
S. equi equi	GGTACTGT	TCGTGTtAAC	GACGAAaTcG	AAATCGTTGG	TATcAgAGAc	GAgATCaAAA	AAGCAGTTGT	TA	218	
S. ferus	GGTACTGT	aaGaGTCAAC	GAtGAAGTTG	AAATCGTTGG	TATcAAAGAc	GAaATCactA	AAGCAGTTGT	TA	219	
S. gordonii	GGTAtcGT	TaaaGTCAAt	GACGAAaTcG	AAATCGTTGG	TATcAAAGAA	GAaATCCAAA	AAGCAGTTGT	TA	220	
S. macacae	GGTACTGT	TaagGTtAAt	GAtGAAGTTG	AAATCGTTGG	TATTcgtGAc	GATATtCAAA	AAGCAGTTGT	TA	222	
S. gordonii	GGTAtcGT	TaaaGTCAAC	GACGAAaTcG	AAATCGTTGG	TATcAAAGAA	GAaActCAAA	AAGCAGTTGT	TA	223	
S. mutans	GGTACTGT	TaaaGTtAAC	GAtGAAGTTG	AAATCGTTGG	TATccgtGAt	GAcATtCAAA	AAGCtGTTGT	TA	224	
S. oralis	GGTACTGT	TCGTGTCAAC	GACGAAaTcG	AAATCGTTGG	TATcAAAGAA	GAaActCAAA	AAGCAGTTGT	TA	-	P33170
S. parasanguinis	GGTgtTGT	TCGTGTCAAt	GAtGAAaTcG	AAATCGTTGG	TATcAAAGAA	GAaATCCAAA	AAGCAGTTGT	TA	225	
S. pneumoniae	GGTAtcGT	TaaaGTCAAC	GACGAAaTcG	AAATCGTTGG	TATcAAAGAA	GAaActCAAA	AAGCAGTTGT	TA	145[a]	
S. pyogenes	GGTACTGT	TCGTGTCAAC	GACGAAaTcG	AAATCGTTGG	TATcAAAGAA	GAaActaAAA	AAGCtGTTGT	TA	-	Genome project
S. ratti	GGTACTGT	TaaaGTCAAt	GACGAAGTTG	AAATCGTTGG	TATccgtGAt	GAcATCCAAA	AAGCtGTTGT	TA	226	
S. salivarius	GGTgtTGT	TCGTGTCAAt	GACGAAGTTG	AAATCGTTGG	TcTTAAAGAA	GAcATCCAAA	AAGCAGTTGT	TA	146[a]	
S. sanguinis	GGTAtcGT	TaaaGTCAAC	GACGAAaTcG	AAATCGTTGG	TATcAAAGAA	GAaATCCAAA	AAGCAGTTGT	TA	227	
S. sobrinus	GGTACTGT	TaagGTtAAC	GACGAAGTTG	AAATCGTTGG	TATccgtGAc	GATATCCAAA	AAGCAGTTGT	TA	228	
S. suis	GGTACTGT	TCGTGTCAAC	GACGAAaTcG	AAATCGTTGG	TcTTcAAGAA	GAaAaatctA	AAGCAGTTGT	TA	229	
S. uberis	GGTACTGT	TCGTGTCAAC	GACGAAaTTG	AAATCGTTGG	TATcAAAGAA	GAaActaAAA	AAGCAGTTGT	TA	230	
S. vestibularis	GGTgtTGT	TCGTGTtAAt	GACGAAGTTG	AAATCGTTGG	TcTTAAAGAA	GAaATCCAAA	AAGCAGTTGT	TA	231	
Selected sequences for species-specific hybridization probes[b]	ACTGT	TCGTGTCAAC	GACGAAGTTG	AAA					582	
				CGTTGG	TATTAAAGAA	GATATCCAAA	AAGCAGTTG		583	

The sequence numbering refers to the *Streptococcus agalactiae tuf* gene fragment (SEQ ID NO. 209). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. Dots indicate gaps in the sequences displayed.

[a] The SEQ ID NO. refers to previous patent publication WO98/20157.
[b] These sequences are the reverse-complement of the selected probes.

Ann x X: *Strategy for the selection of* Streptococcus agalactiae-*specific amplification primers from* atpD *sequences.*

	39			80	203			234	368		399	SEQ ID NO.:
S. agalactiae	TT GATTGTCTAT	AAAAATGGCG	ATAAGTCACA	AAAAGTAGTA..	TAAGGATA	CTTTGGGTCG	TGTCTTCAAC	GTTC..CTT	ATTAGCACCT	TACTTAAAAG	GTGGTAAAG	380
S. agalactiae	TT GATTGTCTAT	AAAAATGGCG	ATAAGTCACA	AAAAGTAGTA..	TAAGGATA	CTTTGGGTCG	TGTCTTCAAC	GTTC..CTT	ATTAGCACCT	TACTTAAAAG	GTGGTAAAG	379
S. agalactiae	TT GATTGTCTAT	AAAAATGGCG	ATAAGTCACA	AAAAGTAGTA..	TAAGGATA	CTTTGGGTCG	TGTCTTCAAC	GTTC..CTT	ATTAGCACCT	TACTTAAAAG	GTGGTAAAG	381
S. agalactiae	TT GATTGTCTAT	AAAAATGGCG	ATAAGTCACA	AAAAGTAGTA..	TAAGGATA	CTTTGGGTCG	TGTCTTCAAC	GTTC..CTT	ATTAGCACCT	TACTTAAAAG	GTGGTAAAG	382
S. agalactiae	TT GATTGTCTAT	AAAAATGGCG	ATAAGTCACA	AAAAGTAGTA..	TAAGGATA	CTTTGGGTCG	TGTCTTCAAC	GTTC..CTT	ATTAGCACCT	TACTTAAAAG	GTGGTAAAG	383
S. bovis	TT GATTGTtTAT	AAAgATGGCG	ATAAGTCtCA	AAAAaTcGTg..	TAAaGAaA	CTTTGGGTCG	TGTgTTtAAt	GTTC..CcT	tcTtGCcCCT	TACcTAAAAG	GTGGTAAAG	-[a]
S. salivarius	TT GgTcGTtTAT	ActgATGaac	AaAAGTCtaA	AcgtaTcGTg..	TAAaGATA	CccTtGGaCG	TGTCTTtAAC	GTTC..CTT	gcTAGCcCCT	TACcTtAAgG	GTGGTAAAG	387
S. pneumoniae	cT tgTcGTCTAc	AAAAATGaCG	AaAgaaaAac	AAAAaTcGTc..	TAAaGAaA	CTTTGGGaCG	TGTCTTCAAC	GTTt..CcT	tcTtGCcCCT	TACcTtAAAG	GTGGTAAAG	-[b]
S. pyogenes	TT GATTGTtTAT	AAAgATaGtG	ATAAaaagCA	AAAAaTcGTc..	TAAaGAaA	CTTTGGGaCG	cGTCTTtAAt	GTaC..CcT	tcTtGCcCCT	TACcTtAAAG	GTGGTAAAG	-[c]
S. anginosus	cT tgTaGTCTAT	AAAAATGaCG	AaAAtaaAtc	AAAAaTcGTc..	gAAaGAaA	CacTtGGTCG	cGTCTTtAAC	GTTt..CcT	tTTAGCcCCc	TACcTcAAAG	GTGGgAAAG	386
S. sanguinis	cT tgTaGTCTAT	AAAAATGatG	AgAAaaaAtc	AAAAaTcGTc..	aAAGGAaA	CTcTaGGcCG	gGTgTTCAAt	GTTt..CcT	gcTAGCACCT	TAtcTgAAAG	GTGGgAAAG	-[d]
S. mutans	TT GgTcGTtTAT	AAAgATGGCG	AcAAGTCtCA	AAgAaTtGTt..	aAAaGAaA	CacTaGGTCG	TGTCTTtAAt	GTTC..CcT	tcTtGCcCCT	TAtcTtAAAG	GTGGTAAAG	-[e]
B. anthracis	gT aAaacagagc	AAcgAaaaCG	gaAcaagcat	tAActTAacA..	TgAtGcaA	CacTtGGTCG	TGTaTTtAAC	GTat..CTT	AcTtGCtCCT	TACaTtAAgG	GTGGTAAga	247
B. cereus	gT aAaacaaagc	AAcgAaaaCG	g...aagcat	gAActTAacA..	TgAtGcaA	CacTtGGaCG	TGTaTTCAAC	GTat..CTT	AcTtGCtCCT	TACaTtAAgG	GTGGTAAga	248
E. faecium	TT agTTGTtTAT	AAAAATGaCG	AaAAtaaAtc	AAAAGTtGTt..	TAAaGAaA	CaTTaGGTCG	cGTaTTCAAC	GTaC..tTT	gcTtGCcCCa	TAtTTAAAAG	GTGGgAAAG	292
E. gallinarum	TT GATcGTtTAc	AAAAAaGaCG	AgAAaaaAac	AAAAGTAGTA..	aAcaGATA	CTcTaGGcCG	aGTaTTtAAt	GTaC..tTT	ATTAGCtCCT	TACTTAAAAG	GTGGTAAAG	293
E. faecalis	TT agTcGTtTAT	AAAAATGGCG	AagcaaaACA	AAAAGTAGTA..	TAAaGATA	CaTTaGGTCG	TGTgTTtAAC	GTTt..CTT	ATTAGCACCT	TAtcTAAAAG	GTGGTAAAG	291
E. coli	Ta cgaTGctctT	gAggtgcaaa	ATggtaatgA	gcgtcTgGTg..	TAAaGcgA	CTcTGGGcCG	TaTCaTgAAC	GTaC..CcT	gaTgtgtCCg	TtCgctAAgG	GcGGTAAAG	-[f]
L. monocytogenes	Ta tAaatctgAT	gcAgAaGaaG	caccaaCtag	ccAAcTtact..	TAcaGtaA	CTcTtGGTCG	TGTaTTtAAt	GTat..CTT	gcTAGCtCCT	TACTTAAAAG	GTGGTAAAa	324
S. aureus	gT tATTGatgtg	cctAAaGaaG	AaggtaCAat	AcAAcTAacA..	TgAtGAaA	CaTTaGGTCG	TGTaTTtAAt	GTaC..tTT	AcTAGCACCT	TAtaTtAAAG	GTGGTAAAa	366
S. epidermidis	ca cATcGaagtT	cctAAaGaaG	ATggagCgCt	tcAAtTAacA..	TgAcGtaA	CTcTaGGaaG	aGTgTTtAAC	GTaC..CTT	ATTAGCACCT	TACaTAAAAG	GTGGTAAAa	370
Selected sequences for species-specific primer	ATTGTCTAT	AAAAATGGCG	ATAAGTC									627
		AAAATGGCG	ATAAGTCACA	AAAAGTA								628
Selected sequences for species-specific primers[g]					GGATA	CTTTGGGTCG	TGTCTTCAAC	G				625
									ATTAGCACCT	TACTTAAAAG	GTGGTA	626

The sequence numbering refers to the *Streptococcus agalactiae tuf* gene fragment (SEQ ID NO. 380). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. Dots indicate gaps in the sequences displayed.

[a,d,e,f] These sequences were obtained from Genbank and have accession #: a=AB009314, d=AF001955, e=U31170, and f=V00311.

[b,c] These sequences were obtained from genome sequencing projects.

[g] These sequences are the reverse-complement of the selected primers.

Annex XI: ***Strategy for the selection of* Candida albicans/dubliniensis*-specific amplification primers,* Candida albicans*-specific hybridization probe and* Candida dubliniensis*-specific hybridization probe from* tuf *sequences.***

	337 … 368 403 … 428 460 … 491	SEQ ID NO.:	Accession #:
C. albicans	CGTC AAGAAGGTTG GTTACAACCC AAAGACTG...CAACATGA TTGAACCATC CACCAACT...C AAATCCGGTA AAGTTACTGG TAAGACCTTG T	624	-
C. albicans	CGTC AAGAAGGTTG GTTACAACCC AAAGACTG...CAACATGA TTGAACCATC CACCAACT...C AAATCCGGTA AAGTTACTGG TAAGACCTTG T	409	-
C. albicans	CGTC AAGAAGGTTG GTTACAACCC AAAGACTG...CAACATGA TTGAACCATC CACCAACT...C AAATCCGGTA AAGTTACTGG TAAGACCTTG T	410	-
C. albicans	CGTC AAGAAGGTTG GTTACAACCC AAAGACTG...CAACATGA TTGAACCATC CACCAACT...C AAATCCGGTA AAGTTACTGG TAAGACCTTG T	407	-
C. albicans	CGTC AAGAAGGTTG GTTACAACCC AAAGACTG...CAACATGA TTGAACCATC CACCAACT...C AAATCCGGTA AAGTTACTGG TAAGACCTTG T	408	-
C. dubliniensis	CGTC AAGAAGGTTG GTTACAACCC AAAGACTG...CAACATGA TTGAAgCtTC CACCAACT...C AAATCCGGTA AgGTTACTGG TAAGACCTTG T	412	-
C. dubliniensis	CGTC AAGAAGGTTG GTTACAACCC AAAGACTG...CAACATGA TTGAAgCtTC CACCAACT...C AAATCCGGTA AgGTTACTGG TAAGACCTTG T	414	-
C. dubliniensis	CGTC AAGAAGGTTG GTTACAACCC AAAGACTG...CAACATGA TTGAAgCtTC CACCAACT...C AAATCCGGTA AgGTTACTGG TAAGACCTTG T	415	-
C. glabrata	CATC AAGAAGGTcG GTTACAACCC AAAGACTG...CAACATGA TTGAAgCcaC CACCAACG...C AAggCtGGTg tcGTcAagGG TAAGACCTTG T	417	-
C. guilliermondii	CGTC AAGAAGGTTG GTTACAACCC tAAGACTG...CAACATGA TTGAggCtTC tACCAACT...C AAggCtGGTA AgtccACcGG TAAGACtTTG T	418	-
C. kefyr	CATC AAGAAGGTcG GTTACAACCC AAAGAATG...CAACATGA TTGAAgCcaC CACCAACG...C AAggCtGGTA ccGTcAagGG TAAGACCTTG T	421	-
C. krusei	CATC AAGAAGGTTG GTTACAACCC AAAGACTG...CAACATGA TTGAAgCATC CACCAACT...C AAggCaGGTg ttGTTAagGG TAAGACCTTA T	422	-
C. lusitaniae	CGTC AAGAAGGTTG GTTACAACCC tAAGACTG...CAACATGA TTGAgCCATC YACCAACT...C AAgTCYGGTA AgtccACcGG TAAGACCTTG T	424	-
C. neoformans	CATC AAGAAGGTTG GTTACAACCC cAAGgCTG...CAACATGt TgGAggagaC CACCAAGT...C AAgTCtGGTg tttccAagGG TAAGACCcTC C	623	-
C. parapsilosis	CGTC AAGAAGGTTG GTTACAACCC tAAagCTG...CAAtATGA TTGAACCATC aACCAACT...T AAAgCtGGTA AgGTTACcGG TAAGACCTTG T	426	-
C. tropicalis	CGTC AAGAAGGTTG GTTACAACCC tAAGgCTG...CAACATGA TTGAAgCtTC tACCAACT...C AAggCtGGTA AgGTTACcGG TAAGACtTTG T	429	-
A. fumigatus	CATC AAGAAGGTcG GcTACAACCC cAAGgCCG...CAACATGc TTGAgCCcTC CtCCAACT...C AAggCCGGcA AgGTcACTGG TAAGACCcTC A	404	-
Human	CATt AAGAAaaTTG GcTACAACCC cgAcACAG...CAACATGc TgGAgCCAag tgCtAACA...T AAggatGGcA AtGccAgTGG aAccACgcTG C	-	X03558
P. anomala	TATC AAGAAaGTTG GTTACAACCC AAAaACTG...TAACATGA TTGAACCATC aWCtAACT...C AAAgCtGGTg AAGcTAaaGG TAAaACtTTA T	447	-
S. cerevisiae	TATC AAGAAGGTTG GTTACAACCC AAAGACTG...CAACATGA TTGAAgCtaC CACCAACG...C AAggCCGGTg tcGTcAagGG TAAGACtTTG T	622	-
S. pombe	CATC AAGAAGGTcG GTTtCAACCC cAAGACCG...TAACATGA TTGAgCCcaC CACCAACA...C AAggCtGGTg tcGTcAagGG TAAGACtcTT T	-	U42189
Selected sequence for species-specific amplification primer[a]	C AAGAAGGTTG GTTACAACCC AAAGA		
Selected sequence for species-specific amplification primer[a,b]	ATCCGGTA AAGTTACTGG TAAGACCT		
Selected sequences for species-specific hybridization probes	CATGA TTGAACCATC CACCA (*C. albicans*)	577	
	CATGA TTGAAGCTTC CACCA (*C. dubliniensis*)	578	

The sequence numbering refers to the *Candida albicans tuf* gene fragment (SEQ ID NO. 408). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches for SEQ ID NO. 577 are indicated by lower-case letters. Mismatches for SEQ ID NO. 578 are indicated by underlined nucleotides. Dots indicate gaps in the sequences displayed. "R" "Y" "M" "K" "W" and "S" designate nucleotide positions which are degenerated. "R" stands for A or G; "Y" stands for C or T; "M" stands for A or C; "K" stands for G or T; "W" stands for A or T; "S" stands for C or G. "I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.

[a] *C. albicans* primers have been described in a previous patent (publication WO98/20157, SEQ ID NOs. 11-12)

[b] This sequence is the reverse-complement of the selected primer.

Annex XII: Strategy for the selection of *Staphylococcus*-specific amplification primers from *tuf* sequences.

	310 ... 340	652 ... 682	SEQ ID NO.:	Accession #:
S. aureus	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	CACTTACCA GAAGGTACTG AAATGGTAAT GC	179	-
S. aureus	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	CACTTACC~ ~~~~~~~~~~ ~~~~~~~~~~ GC	176	-
S. aureus	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	CACTTACCA GAAGGTMCTG AAATGGTAAT GC	177	-
S. aureus aureus	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	CACTTACCA GAAGGTACTG AAATGGTAAT GC	180	-
S. auricularis	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	ActTTACCA GAAGGTACaG AAATGGTAAT GC	181	-
S. capitis capitis	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	AACTTACCA GAAGGTACTG AAATGGTTAT GC	182	-
M. caseolyticus	A CTGGaCGTGT TGAgCGTGGa CAAgTtAAAG...	AACTTACCA GAAGGTACTG AAATGGTAAT GC	183	-
S. cohnii	A CAGGgCGTGT TGAACGTGGT CAAATCAAAG...	ActTTACCA GAAGGTACTG AAATGGTTAT GC	184	-
S. epidermidis	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~	185	-
S. epidermidis	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	AACTTACCA GAAGGTACaG AAATGGTTAT GC	141[a]	-
S. haemolyticus	A CAGGCCGTGT TGAACGTGGg CAAATCAAAG...	AACTTACCA GAAGGTACTG AAATGGTTAT GC	186	-
S. haemolyticus	A CAGGtCGTGT TGAACGTGGT CAAATCAAAG...	AACTTACCA GAAG~~~~~~ ~~~~~~~~~~ ~~	188	-
S. haemolyticus	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	AACTTACCA GAAGGTACTG AAATGG~~~~ ~~	189	-
S. hominis hominis	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	AACTTACCA GAAGGTACTG AAATGGTAAT GC	191	-
S. hominis	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	AACTTACCA GAAGGTACTG AAATGGTAAT GC	193	-
S. hominis	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	AACTTACCA GAAGG~~~~~ ~~~~~~~~~~ ~~	194	-
S. hominis	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	AACTTACCA GAAGGTACTG AAATGGTAAT GC	195	-
S. hominis	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	AACTTACCA GAAGGTACTG AAATGGTAAT GC	196	-
S. lugdunensis	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	AACTTACCA GAAGGTACaG AAATGGTTAT GC	197	-
S. saprophyticus	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~	198	-
S. saprophyticus	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	AACTTACCA GAAGGTACTG AAATGGTTAT GC	199	-
S. saprophyticus	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	AACTTACCA GAAGGTACTG AAATGGTTAT GC	200	-
S. sciuri sciuri	A CAGGCCGTGT TGAACGTGGT CAAATCACTG...	AACTTACCA GAAGGTACTG AAATGGTTAT GC	201	-
S. warneri	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	CAaTTACCA GAAGGTACTG ~~~~~~~~~~ ~~	187	-
S. warneri	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~	192	-
S. warneri	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	CAaTTACCA GAAGGTACTG AAATGGTTAT GC	202	-
B. subtilis	A CTGGCCGTGT aGAACGcGGa CAAgTtAAAG...	CAtcTtCCA GAAGGcgtaG AAATGGTTAT GC	-	Z99104
E. coli	A CCGGtCGTGT aGAACGcGGT atcATCAAAG...	GAacTgCCg GAAGGcgtaG AgATGGTAAT GC	78	-
L. monocytogenes	A CTGGaCGTGT TGAACGTGGa CAAgTtAAAG...	AcacTtCCA GAAGGTACTG AAATGGTAAY GC	138[a]	-
Selected sequence for genus-specific primer	GGCCGTGT TGAACGTGGT CAAATCA		553	
Selected sequences for genus-specific primers[b]		TTACCA GAAGGTACTG AAATGGTIA	575	
		TTACCA GAAGGTACTG AAATGGTWA	707	

The sequence numbering refers to the *Staphylococcus aureus tuf* gene fragment (SEQ ID NO. 179). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. "~" indicate incomplete sequence data. Dots indicate gaps in the sequences displayed.

"R" "Y" "M" "K" "W" and "S" designate nucleotide positions which are degenerated. "R" stands for A or G; "Y" stands for C or T; "M" stands for A or C; "K" stands for G or T; "W" stands for A or T; "S" stands for C or G. "I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.

[a] The SEQ ID NO. refers to previous patent publication WO98/20157.
[b] These sequences are the reverse-complement of the selected primers.

Annex XIII: Strategy for the selection of the *Staphylococcus*-specific hybridization probe from *tuf* sequences.

	400 425	SEQ ID NO.:	Accession #:
S. aureus	G TTGAAATGTT CCGTAAATTA TTAGA	179	-
S. aureus	G TTGAAATGTT CCGTAAATTA TTAGA	176	-
S. aureus	G TTGAAATGTT CCGTAAATTA TTAGA	177	-
S. aureus	G TTGAAATGTT CCGTAAATTA TTAGA	178	-
S. aureus aureus	G TTGAAATGTT CCGTAAATTA TTAGA	180	-
S. auricularis	G TAGAAATGTT CCGTAAATTA TTAGA	181	-
S. capitis capitis	G TAGAAATGTT CCGTAAATTA TTAGA	182	-
M. caseolyticus	G TAGAAATGTT CCGTAAATTA TTAGA	183	-
S. cohnii	G TAGAAATGTT CCGTAAATTA TTAGA	184	-
S. epidermidis	G TAGAAATGTT CCGTAAATTA TTAGA	185	-
S. haemolyticus	G TAGAAATGTT CCGTAAATTA TTAGA	186	-
S. haemolyticus	G TAGAAATGTT CCGTAAATTA TTAGA	189	-
S. haemolyticus	G TAGAAATGTT CCGTAAATTA TTAGA	190	-
S. haemolyticus	G TAGAAATGTT CCGTAAATTA TTAGA	188	-
S. hominis	G TAGAAATGTT CCGTAAATTA TTAGA	196	-
S. hominis	G TAGAAATGTT CCGTAAATTA TTAGA	194	-
S. hominis hominis	G TAGAAATGTT CCGTAAATTA TTAGA	191	-
S. hominis	G TAGAAATGTT CCGTAAATTA TTAGA	193	-
S. hominis	G TAGAAATGTT CCGTAAATTA TTAGA	195	-
S. lugdunensis	G TAGAAATGTT CCGTAAATTA TTAGA	197	-
S. saprophyticus	G TAGAAATGTT CCGTAAATTA TTAGA	198	-
S. saprophyticus	G TAGAAATGTT CCGTAAATTA TTAGA	200	-
S. saprophyticus	G TAGAAATGTT CCGTAAATTA TTAGA	199	-
S. sciuri sciuri	G TTGAAATGTT CCGTAAATTA TTAGA	201	-
S. warneri	G TAGAAATGTT CCGTAAgTTA TTAGA	187	-
S. warneri	G TAGAAATGTT CCGTAAgTTA TTAGA	192	-
S. warneri	G TAGAAATGTT CCGTAAgTTA TTAGA	202	-
S. warneri	G TAGAAATGTT CCGTAAgTTA TTAGA	203	-
B. subtilis	G TTGAAATGTT CCGTAAgcTt cTTGA	-	Z99104
E. coli	G TTGAAATGTT CCGcAAAcTg cTGGA	78	-
L. monocytogenes	G TAGAAATGTT CCGTAAATTA cTAGA	138[a]	-
Selected sequence for genus-specific hybridization probe	GAAATGTT CCGTAAATTA TT	605	

The sequence numbering refers to the *Staphylococcus aureus tuf* gene fragment (SEQ ID NO. 179). Nucleotides in capitals are identical to the selected sequence or match that sequence. Mismatches are indicated by lower-case letters.

[a] The SEQ ID NO. refers to previous patent publication WO98/20157.

Annex XIV: Strat gy for the s lection of *Staphylococcus saprophyticus*-sp cific and of *Staphylococcus haemolyticus*-specific hybridization probes from *tuf* sequences.

	339					383	SEQ ID NO.:
S. aureus	AG	TtGGTGAAGA	AgTtGAAATC	ATcGGTtTaC	ATGACACaTC	TAA	179
S. aureus	AG	TtGGTGAAGA	AgTtGAAATC	ATcGGTtTaC	ATGACACaTC	TAA	176
S. aureus	AG	TtGGTGAAGA	AgTtGAAATC	ATcGGTtTaC	ATGACACaTC	TAA	177
S. aureus	AG	TtGGTGAAGA	AgTtGAAATC	ATcGGTtTaC	ATGACACaTC	TAA	178
S. aureus aureus	AG	TtGGTGAAGA	AgTtGAAATC	ATcGGTtTaC	ATGACACaTC	TAA	180
S. auricularis	AG	TCGGTGAAGA	AgTtGAAATC	ATcGGTATga	AaGACggTTC	AAA	181
S. capitis capitis	AG	TtGGTGAAGA	AgTtGAAATC	ATcGGTATCC	AcGAaACTTC	TAA	182
M. caseolyticus	AG	TtGGTGAAGA	AgTtGAAATC	ATTGGTtTaa	cTGAagaacC	AAA	183
S. cohnii	AG	TCGGTGAAGA	AgTtGAAATC	ATcGGTATgC	AaGAagaTTC	CAA	184
S. epidermidis	AG	TtGGTGAAGA	AgTtGAAATC	ATcGGTATgC	AcGAaACTTC	TAA	185
S. haemolyticus	AG	TtGGTGAAGA	AgTtGAAATC	ATTGGTATCC	ATGACACTTC	TAA	186
S. haemolyticus	AG	TtGGTGAAGA	AgTtGAAATC	ATTGGTATCC	ATGACACTTC	TAA	189
S. haemolyticus	AG	TtGGTGAAGA	AgTtGAAATC	ATTGGTATCC	ATGACACTTC	TAA	190
S. haemolyticus	AG	TtGGTGAAGA	AgTtGAAATt	ATTGGTATCa	AaGAaACTTC	TAA	188
S. haemolyticus	AG	TtGGTGAAGA	AgTtGAAATt	ATTGGTATCa	AaGAaACTTC	TAA	194
S. hominis	AG	TtGGTGAAGA	AgTtGAAATt	ATTGGTATCa	AaGAaACTTC	TAA	191
S. hominis hominis	AG	TtGGTGAAGA	AgTtGAAATt	ATTGGTATCa	AaGAaACTTC	TAA	193
S. hominis	AG	TtGGTGAAGA	AgTtGAAATt	ATTGGTATCa	AaGAaACTTC	TAA	195
S. hominis	AG	TtGGTGAAGA	AgTtGAAATt	ATTGGTATCa	AaGAtACTTC	TAA	196
S. lugdunensis	AG	TCGGTGAAGA	AgTtGAAATt	ATTGGTATCC	AcGAtACTaC	TAA	197
S. saprophyticus	AG	TCGGTGAAGA	AATCGAAATC	ATcGGTATgC	AaGAagaaTC	CAA	198
S. saprophyticus	AG	TCGGTGAAGA	AATCGAAATC	ATcGGTATgC	AaGAagaaTC	CAA	200
S. saprophyticus	AG	TCGGTGAAGA	AATCGAAATC	ATcGGTATgC	AaGAagaaTC	CAA	199
S. sciuri sciuri	TG	TtGGTGAAGA	AgTtGAAATC	ATcGGTtTaa	cTGAagaaTC	TAA	201
S. warneri	AG	TtGGTGAAGA	AgTtGAAATC	ATcGGTtTaC	ATGACACTTC	TAA	187
S. warneri	AG	TtGGTGAAGA	AgTtGAAATC	ATcGGTtTaC	ATGACACTTC	TAA	192
S. warneri	AG	TtGGTGAAGA	AgTtGAAATC	ATcGGTtTaC	ATGACACTTC	TAA	202
S. warneri	AG	TtGGTGAAGA	AgTtGAAATC	ATcGGTtTaC	ATGACACTTC	TAA	203
B. subtilis	AG	TCGGTGAcGA	AgTtGAAATC	ATcGGTcTtC	AaGAagagag	AAA	–[a]
E. coli	AG	TtGGTGAAGA	AgTtGAAATC	gTTGGTATCa	AaGAgACTca	GAA	78
L. monocytogenes	AG	TtGGTGAcGA	AgTaGAAgTt	ATcGGTATCg	AaGAagaaag	AAA	138[b]

Selected sequences for species-specific hybridization probes	**CGGTGAAGA AATCGAAATC A (*S. saprophyticus*)**	599
	(*S. haemolyticus*) ATTGGTATCC ATGACACTTC	594

The sequence numbering refers to the *Staphylococcus aureus tuf* gene fragment (SEQ ID NO. 179). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters.

[a] This sequence was obtained from Genbank accession #Z99104.
[b] The SEQ ID NO. refers to previous patent publication WO98/20157.

Annex XV: Strategy for the selection of *Staphylococcus aureus*-specific and of *Staphylococcus epidermidis*-specific hybridization probes from *tuf* sequences.

	521 ... 547	592 ... 617	SEQ ID NO.:
S. aureus	TACACCACA TACTGAATTC AAAGCAG...	...TTCTTCtCa AACTATCGtC CACAATT	179
S. aureus	TACACCACA TACTGAATTC AAAGCAG...	...TTCTTCtC- ---------- -------	178
S. aureus	TACACCACA TACTGAATTC AAAGCAG...	...TTCTTCtCa AACTATCGtC CACAATT	176
S. aureus	TACACCACA TACTGAATTC AAAGCAG...	...TTCTTCtCa AACTATCGtC CACAATT	177
S. aureus aureus	TACACCACA TACTGAATTC AAAGCAG...	...TTCTTCtCa AACTATCGtC CACAATT	180
S. auricularis	TACACCACA cACTaAATTC ActGCAG...	...TTCTTCtCT AACTAcCGtC CACAATT	181
S. capitis capitis	CACACCACA cACTaAATTC AAAGCGG...	...TTCTTCAgT AACTAcCGCC CACAATT	182
M. caseolyticus	TACtCCACA TACTaAATTC AAAGCTG...	...TTCTTCACT AACTAcCGCC CtCAGTT	183
S. cohnii	TACACCACA cACaaAcTTt AAAGCGG...	...TTCTTCAgT AACTATCGCC CACAATT	184
S. epidermidis	TACACCACA cACaaAATTC AAAGCTG...	...TTCTTCACT AACTATCGCC CACAATT	185
S. haemolyticus	CACACCtCA cACaaAATTt AAAGCAG...	...TTCTTCACa AACTATCGtC CACAATT	186
S. haemolyticus	CACACCtCA cACaaAATTt AAAGCAG...	...TTCTTCACa AACTATCGtC CACAATT	189
S. haemolyticus	CACACCtCA cACaaAATTt AAAGCAG...	...TTCTTCACa AACTATCGtC CACAATT	190
S. haemolyticus	TACACCtCA cACaaAATTC AAAGCAG...	...TTCTTCACT AACTATCGtC CACAATT	188
S. hominis	CACACCtCA cACaaAATTC AAAGCAG...	...TTCTTCACT AACTATCGtC CACAATT	195
S. hominis	TACACCtCA cACaaAATTC AAAGCAG...	...TTCTTCACT AACTATCGtC CACAATT	196
S. hominis hominis	TACACCtCA cACaaAATTC AAAGCAG...	...TTCTTCtCT AACTATCGtC CACAATT	191
S. hominis	TACACCtCA cACaaAATTC AAAGCAG...	...TTCTTCtCT AACTATCGtC CACAATT	193
S. hominis	TACACCtCA cACaaAATTC AAAGCAG...	...TTCTTCtCT AACTATCGtC CACAATT	194
S. lugdunensis	TACACCtCA cACTaAATTt AAAGCTG...	...TTCTTCtCa AACTAcCGCC CACAATT	197
S. saprophyticus	TACACCACA TACaaAATTC AAAGCGG...	...TTCTTCACT AACTAcCGCC CACAATT	198
S. saprophyticus	TACACCACA TACaaAATTC AAAGCGG...	...TTCTTCACT AACTAcCGCC CACAATT	199
S. saprophyticus	TACACCACA TACaaAATTC AAAGCGG...	...TTCTTCACT AACTAcCGCC CACAATT	200
S. sciuri sciuri	CACACCtCA cACTaAATTC AAAGCTG...	...TTCTTCACa AACTAcCGCC CACAATT	201
S. warneri	TACACCACA TACaaAATTC AAAGCGG...	...--------- ---------- -------	192
S. warneri	TACACCACA TACaaAATTC AAAGCGG...	...TTCTTCAgT AACTAcCGCC CACAATT	187
S. warneri	TACACCACA TACaaAATTC AAAGCGG...	...TTCTTCAgT AACTAcCGCC CACAATT	202
S. warneri	TACACCACA TACaaAATTC AAAGCGG...	...TTCTTCAgT AACTAcCGCC CACAATT	203
B. subtilis	CACtCCACA cAgcaAATTC AAAGCTG...	...TTCTTCtCT AACTAcCGtC CtCAGTT	-[a]
E. coli	CAAgCCgCA cACcaAgTTC gAAtCTG...	...TTCTTCAaa ggCTAcCGtC CgCAGTT	78
L. monocytogenes	TACtCCACA cACTaAcTTC AAAGCTG...	...TTCTTCAac AACTAcCGCC CACAATT	138[b]
Selected sequences for species-specific hybridization probes	ACCACA TACTGAATTC AAAG (*S. aureus*)		585
		(*S. epidermidis*) TTCACT AACTATCGCC CACA	593

The sequence numbering refers to the *Staphylococcus aureus tuf* gene fragment (SEQ ID NO. 179). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. "-" indicate incomplete sequence data. Dots indicate gaps in the sequences displayed.

[a] This sequence was obtained from Genbank accession #Z99104.
[b] The SEQ ID NO. refers to previous patent publication WO98/20157.

Annex XVI: Strategy for the selection of the *Staphylococcus hominis*-specific hybridization probe from *tuf* sequences.

	358 383	SEQ ID NO.:
S. aureus	ATC **ATcGGTtTac AtGAcACaTC** TAA	179
S. aureus	ATC **ATcGGTtTac AtGAcACaTC** TAA	176
S. aureus	ATC **ATcGGTtTac AtGAcACaTC** TAA	177
S. aureus	ATC **ATcGGTtTac AtGAcACaTC** TAA	178
S. aureus aureus	ATC **ATcGGTtTac AtGAcACaTC** TAA	180
S. auricularis	ATC **ATcGGTATgA AAGAcggTTC** AAA	181
S. capitis capitis	ATC **ATcGGTATCc AcGAAACTTC** TAA	182
M. caseolyticus	ATC **ATTGGTtTaA ctGAAgaacC** AAA	183
S. cohnii	ATC **ATcGGTATgc AAGAAgaTTC** CAA	184
S. epidermidis	ATC **ATcGGTATgc AcGAAACTTC** TAA	185
S. haemolyticus	ATC **ATTGGTATCc AtGAcACTTC** TAA	186
S. haemolyticus	ATC **ATTGGTATCc AtGAcACTTC** TAA	189
S. haemolyticus	ATC **ATTGGTATCc AtGAcACTTC** TAA	190
S. haemolyticus	ATT **ATTGGTATCA AAGAAACTTC** TAA	188
S. hominis	ATT **ATTGGTATCA AAGAtACTTC** TAA	196
S. hominis	ATT **ATTGGTATCA AAGAAACTTC** TAA	194
S. hominis hominis	ATT **ATTGGTATCA AAGAAACTTC** TAA	191
S. hominis	ATT **ATTGGTATCA AAGAAACTTC** TAA	193
S. hominis	ATT **ATTGGTATCA AAGAAACTTC** TAA	195
S. lugdunensis	ATT **ATTGGTATCc AcGAtACTaC** TAA	197
S. saprophyticus	ATC **ATcGGTATgc AAGAAgaaTC** CAA	198
S. saprophyticus	ATC **ATcGGTATgc AAGAAgaaTC** CAA	200
S. saprophyticus	ATC **ATcGGTATgc AAGAAgaaTC** CAA	199
S. sciuri sciuri	ATC **ATcGGTtTaA ctGAAgaaTC** TAA	201
S. warneri	ATC **ATcGGTtTac AtGAcACTTC** TAA	187
S. warneri	ATC **ATcGGTtTac AtGAcACTTC** TAA	192
S. warneri	ATC **ATcGGTtTac AtGAcACTTC** TAA	202
S. warneri	ATC **ATcGGTtTac AtGAcACTTC** TAA	203
B. subtilis	ATC **ATcGGTcTtc AAGAAgagag** AAA	_[a]
E. coli	ATC **gTTGGTATCA AAGAgACTca** GAA	78
L. monocytogenes	GTT **ATcGGTATCg AAGAAgaaag** AAA	138[b]
Selected sequence for species-specific hybridization probe	**ATTGGTATCA AAGAAACTTC**	597

The sequence numbering refers to the *Staphylococcus aureus tuf* gene fragment (SEQ ID NO. 179). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. Dots indicate gaps in the sequences displayed.

[a] This sequence was obtained from Genbank accession #Z99104.
[b] The SEQ ID NO. refers to previous patent publication WO98/20157.

Ann x XVII: Strategy for the selection of the *Enterococcus*-specific amplification primers from *tuf* sequences.

	270 298	556 582	SEQ ID NO.:	Accession #:
E. avium	TAGAATTAAT GGCTGCTGTT GACGAATAT...	TGAA GATATCCAAC GTGGACAAGT ATT	131[a]	-
E. casseliflavus	TGGAATTAAT GGCTGCAGTT GACGAATAC...	TGAA GACATCCAAC GTGGACAAGT ATT	58	-
E. cecorum	TAGAATTAAT GGCTGCAGTT GACGAATAC...	TGAA GATATCCAAC GTGGtCAAGT ATT	59	-
E. dispar	TAGAATTAAT GGCTGCAGTT GACGAATAT...	TGAA GATATCCAAC GTGGtCAAGT ATT	60	-
E. durans	TTGAATTAAT GGCTGCAGTT GACGAATAT...	TGAA GACATCCAAC GTGGACAAGT TTT	61	-
E. flavescens	TGGAATTAAT GGCTGCAGTT GACGAATAC...	TGAA GACATCCAAC GTGGACAAGT ATT	65	-
E. faecium	TTGAATTAAT GGCTGCAGTT GACGAATAC...	TGAA GACATCCAAC GTGGACAAGT TTT	608	-
E. faecalis	TAGAATTAAT GGCTGCAGTT GACGAATAT...	TGAA GATATCGAAC GTGGACAAGT ATT	607	-
E. gallinarum	TGGAATTgAT GGCTGCAGTT GACGAATAC...	TGAA GACATCCAAC GTGGACAAGT ATT	609	-
E. hirae	TTGAATTgAT GGCTGCAGTT GACGAATAT...	TGAA GACATCCAAC GTGGACAAGT TTT	67	-
E. mundtii	TTGAATTgAT GGCTGCAGTT GACGAATAT...	TGAA GACATCCAAC GTGGtCAAGT TTT	68	-
E. pseudoavium	TAGAATTAAT GSCTGCTGTT GACGAATAC...	TGAA GACATCCAAC GTGGACAAGT ATT	69	-
E. raffinosus	TAGAATTAAT GGCTGCTGTT GATGAATAC...	TGAA GACATCCAAC GTGGACAAGT ATT	70	-
E. saccharolyticus	TCGAATTAAT GGCTGCAGTT GACGAATAT...	TGAA GACATCCAAC GTGGACAAGT ATT	71	-
E. solitarius	TGGAcTTAAT GGaTGCAGTT GATGAcTAC...	TGAt GATATCGAAC GTGGtCAAGT ATT	72	-
E. coli	TGGAAcTggc tGgcttccTg GATtctTAY...	TGAA GAaATCGAAC GTGGtCAgGT ACT	78	-
B. cepacia	TGAgccTggc cGacGCgcTg GACacgTAC...	TGAA GACgTgGAgC GTGGcCAgGT TCT	16	-
B. fragilis	TGGAAcTgAT GGaaGCTGTT GATactTGG...	GAAc GAaATCaAAC GTGGtatgGT TCT	-	M22247
B. subtilis	TCGAAcTtAT GGaTGCgGTT GATGAgTAC...	TGAA GAaATCCAAC GTGGtCAAGT ACT	-	Z99104
C. diphtheriae	TCGAccTcAT GcagGCTtgc KATGAtTCC...	CGAA GACgTtGAgC GTGGcCAgGT TGT	662	-
C. trachomatis	GAGAgcTAAT GcaaGCcGTc GATGAtAAT...	GAAc GATgTgGAAa GaGGAatgGT TGT	22	-
G. vaginalis	AGGAAcTcAT GaagGCTGTT GACGAgTAC...	TACc GACgTtGAgC GTGGtCAgGT TGT	135[a]	-
S. aureus	TAGAATTART GGaaGCTGTa GATactTAC...	TGAA GACgTaCAAC GTGGtCAAGT ATT	179	-
S. pneumoniae	TGGAATTgAT GaacaCAGTT GATGAgTAT...	TGAt GAaATCGAAC GTGGACAAGT TAT	145[a]	-
A. adiacens	TAGAATTAAT GGCTGCTGTT GACGAATAC...	TGAA aACATCGAAC GTGGACAAGT TCT	118[a]	-
G. haemolysans	TCGAATTAAT GGaaaCAGTT GACGAATAC...	TGAA GACATCGAAC GTGGACAAGT TTT	87	-
G. morbillorum	TCGAATTAAT GGaaaCAGTT GACGAgTAC...	TGAA GATATCGAAC GTGGACAAGT TTT	88	-
Selected sequence for amplification primer	AATTAAT GGCTGCWGTT GAYGAA		1137	
Selected sequence for amplification primer[b]		A GAYATCSAAC GTGGACAAGT	1136	

The sequence numbering refers to the *Enterococcus durans tuf* gene fragment (SEQ ID NO. 61). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. Dots indicate gaps in the sequences displayed.
"Y" "W" and "S" designate nucleotide positions which are degenerated. "Y" stands for C or T; "W" stands for A or T; "S" stands for C or G. "I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.

[a] The SEQ ID NO. refers to previous patent publication WO98/20157.
[b] This sequence is the reverse-complement of the selected primer.

Ann x XVIII: Strategy for the selection of the *Enterococcus faecalis*-specific hybridization probe, of the *Enterococcus faecium*-specific hybridization probe and of the *Enterococcus casseliflavus-flavescens-gallinarum* group-specific hybridization probe from *tuf* sequences.

	395 ... 448...526 ... 549	SEQ ID NO.:	Accession #:
E. avium	GTTGA ACGTGGacAA GTTCGCGTTG GTGACGAAGT TGAAaTcGTa GGTATcGCT...CATc GGTGCtTTGt TACGTGGTGT	131[a]	-
E. casseliflavus	GTTGA ACGTGGacAA GTTCGCGTTG GTGACGAAGT TGAAaTcGTT GGTATTGCT...CATT GGTGCATTGC TACGTGGTGT	58	-
E. cecorum	GTTGA ACGTGGacAA GTaCGtGTTG GTGACGAAGT TGAAaTaGTT GGTATcCAT...CATc GGTGCATTat TACGTGGTGT	59	-
E. dispar	GTTGA ACGTGGacAA GTTCGCGTTG GTGACGAAGT TGAAaTcGTa GGTATcGCT...CATT GGTGCATTat TACGTGGTGT	60	-
E. durans	GTTGA ACGTGGacAA GTTCGCGTTG GTGACGttGT aGAtaTcGTT GGTATcGCA...CATT GGTGCtTTaC TACGTGGTGT	61	-
E. faecalis	GTTGA ACGTGGTGAA GTTCGCGTTG GTGACGAAGT TGAAaTcGTT GGTATTAAA...CTTc GGTGCtTTat TACGTGGTGT	62	-
E. faecium	GTTGA ACGTGGacAA GTTCGCGTTG GTGACGAAGT TGAAGTTGTT GGTATTGCT...CATT GGTGCtTTaC TACGTGGTGT	608	-
E. flavescens	GTTGA ACGTGGacAA GTTCGCGTTG GTGACGAAGT TGAAaTcGTT GGTATTGCT...CATT GGTGCATTGC TACGTGGGGT	65	-
E. gallinarum	GTTGA ACGTGGacAA GTTCGCGTTG GTGATGAAGT aGAAaTcGTT GGTATTGCT...CATT GGTGCATTGC TACGTGGGGT	609	-
E. hirae	GTTGA ACGTGGacAA GTTCGCGTTG GTGACGttGT aGAtaTcGTT GGTATcGCA...CATT GGTGCtTTaC TACGTGGTGT	67	-
E. mundtii	GTTGA ACGTGGacAA GYTCGtGTTG GTGACGttaT cGAtaTcGTT GGTATcGCA...CATT GGTGCgTTaC TACGTGGTGT	68	-
E. pseudoavium	GTTGA ACGTGGacAA GTTCGCGTTG GTGACGAAGT TGAAaTcGTa GGTATCGCT...CATc GGTGCATTat TACGTGGTGT	69	-
E. raffinosus	GTTGA ACGTGGacAA GTTCGCGTTG GTGACGAAGT TGAAaTcGTa GGTATTGCT...CATT GGTGCATTat TACGTGGTGT	70	-
E. saccharolyticus	GTTGA ACGTGGacAA GTTCGCGTTG GTGACGttGT aGAAaTcGTT GGTATcGAC...CATc GGTGCtTTat TACGTGGGGT	71	-
E. solitarius	GTTGA ACGcGGact aTcaaaGTCG GCGATGAAGT TGAcaTTaTT GGTATTCAT...CATT GGTaCtTTGt TACGTGGTGT	72	-
C. diphtheriae	GTTGA gCGTGGctcc cTgaagGTCA ACGAGGAcGT cGAgaTcaTc GGTATcCGC...CTGT GGTctgcTtC TcCGTGGCGT	662	-
G. vaginalis	GTTGA gCGTGGTaAg cTcCcaATCA ACACCCcAGT TGAgaTcGTT GGTtTgCGC...CACT GGTcttcTtC TcCGcGGTAT	135[a]	-
B. cepacia	GTCGA gCGcGGcatc GTgaagGTCG GCGAAGAAaT cGAAaTcGTc GGTATcAAG...CGTT GGTatccTGC TgCGcGGCAC	16	-
S. aureus	GTTGA ACGTGGTcAA aTcaaaGTTG GTGAAGAAGT TGAAaTcaTc GGTtTaCAT...CATT GGTGCATTat TACGTGGTGT	179	-
B. subtilis	GTAGA ACGcGGacAA GTTaaaGTCG GTGACGAAGT TGAAaTcaTc GGTcTTCAA...CATT GGTGCccTtC TtCGcGGTGT	-	Z99104
S. pneumoniae	ATCGA cCGTGGTatc GTTaaaGTCA ACGACGAAaT cGAAaTcGTT GGTATcAAA...CGTa GGTGtccTtC TtCGTGGTGT	145[a]	-
E. coli	GTAGA ACGcGGTatc aTcaaaGTTG GTGAAGAAGT TGAAaTcGTT GGTATcAAA...CGTa GGTGttcTGC TgCGTGGTAT	78	-
B. fragilis	ATCGA AacTGGTGtt aTcCatGTAG GTGATGAAaT cGAAaTccTc GGTtTgGGT...CGTa GGTctgTTGC TtCGTGGTGT	-	M22247
C. trachomatis	ATTGA gCGTGGaatt GTTaaaGTTT CCGATAAAGT TcAgtTgGTc GGTcTTAGA...CGTT GGattgcTcC TcaGaGGTAT	22	-
Selected sequences for species-specific or group-specific hybridization probes	GA ACGTGGTGAA GTTCGC (*E. faecalis*)	1174	
	AAGT TGAAGTTGTT GGTATT (*E. faecium*)	602	
	T GGTGCATTGC TACGTGG	1122	

The sequence numbering refers to the *Enterococcus faecium tuf* gene fragments (SEQ ID NO. 608). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. Dots indicate gaps in the sequences displayed.

[a] The SEQ ID NO. refers to previous patent publication WO98/20157.

Annex XIX: Strategy for the selection of primers for the identification of platelets contaminants from *tuf* sequences.

	467 ... 495	689 ... 717	SEQ ID NO.:	Accession #:
B. cereus	GTA ACTGGTGTaG AGATGTTCCG TAAACT...	C AGTTCTACTT CCGTACAACT GACGTAAC	7	-
B. subtilis	GTT ACaGGTGTTG AAATGTTCCG TAAGCT...	C AGTTCTACTT CCGTACAACT GACGTAAC	-	Z99104
E. cloacae	TGT ACTGGCGTTG AAATGTTCCG CAAACT...	C AGTTCTACTT CCGTACAACT GACGTGAC	54	-
E. coli	TGT ACTGGCGTTG AAATGTTCCG CAAACT...	C AGTTCTACTT CCGTACTACT GACGTGAC	78	-
K. oxytoca	TGT ACTGGCGTTG AAATGTTCCG CAAACT...	C AGTTCTACTT CCGTACAACT GACGTGAC	100	-
K. pneumoniae	TGT ACTGGCGTTG AAATGTTCCG CAAACT...	C AGTTCTACTT CCGTACTACT GACGTGAC	103	-
P. aeruginosa	TGC ACcGGCGTTG AAATGTTCCG CAAGCT...	C AGTTCTACTT CCGTACCACK GACGTGAC	153	-
S. agalactiae	GTT ACTGGTGTTG AAATGTTCCG TAAACA...	C AATTCTACTT CCGTACAACT GACGTAAC	209	-
S. aureus	GTT ACaGGTGTTG AAATGTTCCG TAAATT...	C AATTCTATTT CCGTACTACT GACGTAAC	140[a]	-
S. choleraesuis	TGT ACTGGCGTTG AAATGTTCCG CAAACT...	C AGTTCTACTT CCGTACTACT GACGTGAC	159	-
S. epidermidis	GTT ACTGGTGTaG AAATGTTCCG TAAATT...	C AATTCTATTT CCGTACTACT GACGTAAC	611	-
S. marcescens	TGT ACTGGCGTTG AAATGTTCCG CAAACT...	C AGTTCTACTT CCGTACCACT GACGTGAC	168	-
S. mutans	GTT ACTGGTGTTG AAATGTTCCG TAAACA...	C AATTCTACTT CCGTACAACT GACGTAAC	224	-
S. pyogenes	GTT ACTGGTGTTG AAATGTTCCG TAAACA...	C AATTCTACTT CCGTACAACT GACGTAAC	-	U40453
S. salivarius	GTT ACTGGTGTTG AAATGTTCCG TAAACA...	C AGTTCTACTT CCGTACAACT GACGTAAC	146[a]	-
S. sanguinis	GTT ACTGGTGTTG AAATGTTCCG TAAACA...	C AGTTCTACTT CCGTACAACT GACGTTAC	227	-
Y. enterocolitica	TGT ACTGGCGTTG AAATGTTCCG CAAACT...	C AGTTCTACTT CCGTACAACT GAtGTAAC	235	-
Selected sequence for amplification primer	ACTGGYGTTG AIATGTTCCG YAA		636	
Selected sequence for amplification primer[b]		TTCTAYTT CCGTACIACT GACGT	637	

The sequence numbering refers to the *E. coli tuf* gene fragment (SEQ ID NO. 78). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. Dots indicate gaps in the sequences displayed.

"R" "Y" "M" "K" "W" and "S" designate nucleotide positions which are degenerated. "R" stands for A or G; "Y" stands for C or T; "M" stands for A or C; "K" stands for G or T; "W" stands for A or T; "S" stands for C or G. "I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.

[a] The SEQ ID NO. refers to previous patent publication WO98/20157.
[b] This sequence is the reverse-complement of the selected primer.

Annex XX: Strategy for the selection of the universal amplification primers from *atpD* sequences.

	616 – 657 781 – 812	SEQ ID NO.:	Accession #:
C. glutamicum	GTGTTCGGTC AGATGGATGA GCCACCAGGA GTCCGTATG CGC...CGTATg CCTTCCGCCG TGGGTTACCA GCCAAC	-	X76875
M. tuberculosis	GTATTCGGAC AGATGGACGA GCCGCCGGGC aCCCGTATG CGT...CGGATg CCGTCGGCCG TGGGATACCA GCCCAC	-	Z73419
E. faecalis	GTGTTCGGAC AAATGAACGA ACCACCAGGT GCTCGGATG CGG...CGTATg CCTTCTGCCG TTGGTTACCA ACCAAC	291	-
S. agalactiae	GTCTTTGGTC AAATGAATGA ACCACCAGGA GCACGTATG CGT...CGTATg CCTTCAGCCG TTGGTTATCA ACCAAC	380	-
B. subtilis	GTATTCGGAC AAATGAACGA GCCGCCGGGC GCACGTATG CGT...CGTATg CCTTCAGCGG TTGGTTATCA GCCGAC	-	Z28592
L. monocytogenes	GTATTCGGTC AAATGAACGA GCCACCAGGT GCGCGTATG CGT...CGTATg CCATCTGCGG TAGGTTACCA ACCAAC	324	-
S. aureus	GTATTCGGGC AAATGAATGA GCCACCTGGT GCACGTATG CGT...CGTATg CCTTCTGCAG TAGGTTACCA ACCAAC	366	-
A. baumannii	GTCTACGGTC AGATGAACGA GCCACCAGGT aaCCGTtTa CGC...CGTATg CCATCTGCGG TAGGTTACCA ACCTAC	243	-
N. gonorrhoeae	GTGTATGGCC AAATGAACGA ACCTCCAGGC aaCCGTcTG CGC...CGTATg CCTTCTGCAG TGGGTTACCA ACCGAC	-	Genome project
C. freundii	GTATATGGCC AGATGAACGA GCCGCCTGGA aaCCGTcTG CGT...CGTATg CCATCAGCGG TAGGCTACCA GCCGAC	264	-
E. cloacae	GTTTACGGCC AGATGAACGA GCCACCAGGA aaCCGTcTG CGC...CGTATg CCTTCAGCGG TAGGTTATCA GCCTAC	284	-
E. coli	GTGTATGGCC AGATGAACGA GCCGCCGGGA aaCCGTcTG CGC...CGTATg CCTTCAGCGG TAGGTTATCA GCCGAC	669	V00267
S. typhimurium	GTGTATGGCC AGATGAACGA GCCGCCGGGA aaCCGTcTG CGC...CGTATg CCTTCCGCAG TAGGTTACCA GCCGAC	351	-
K. pneumoniae	GTGTACGGCC AGATGAACGA GCCGCCGGGA aaCCGTcTG CGC...CGTATg CCTTCAGCGG TAGGTTATCA GCCGAC	317	-
S. marcescens	GTTTACGGCC AGATGAACGA GCCACCAGGT aaCCGTcTG CGC...CGTATg CCATCCGCGG TAGGTTATCA GCCAAC	357	-
Y. enterocolitica	GTTTATGGCC AAATGAATGA GCCACCAGGT aaCCGTcTG CGC...CGTATg CCATCTGCCG TAGGTTACCA GCCAAC	393	-
B. cepacia	GTGTACGGCC AGATGAACGA GCCGCCGGGC aaCCGTcTG CGC...CGTATg CCGTCGGCAG TGGGCTATCA GCCGAC	-	X76877
H. influenzae	GTTTATGGTC AAATGAACGA GCCACCAGGT aaCCGTtTa CGT...CGTATg CCATCCGCGG TAGGTTACCA ACCGAC	-	U32730
M. pneumoniae	GTGTTTGGTC AGATGAACGA ACCCCCAGGA GCACGGATG CGG...CGGATg CCATCAGCCG TGGGTTACCA ACCAAC	-	U43738
H. pylori	TGCTATGGGC AAATGAATGA GCCACCAGGT GCAAGGAAt CGC...CGTATC CCTTCAGCGG TGGGGTATCA GCCCAC	670	V00267
B. fragilis	GTGTTCGGAC AGATGAACGA ACCTCCTGGA GCACGTgct TCA...CGTATg CCTTCTGCGG TAGGTTATCA ACCTAC	-	M22247
Selected sequences for universal primers	C ARATGRAYGA RCCICCIGGI GYIMGIATG	562	
	TAYGGIC ARATGAAYGA RCCICCIGGI AA	564	
Selected sequences for universal primers[a]	ATH CCITCIGCIG TIGGITAYCA RCC	565	
	ATG CCITCIGCIG TIGGITAYCA RCC	563	

The sequence numbering refers to the *Escherichia coli atpD* gene fragment (SEQ ID NO. 669). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches for SEQ ID NOs. 562 and 565 are indicated by lower-case letters. Mismatches for SEQ ID NOs. 564 and 563 are indicated by underlined nucleotides. Dots indicate gaps in the sequences displayed.

"R" "Y" "M" "K" "W" and "S" letters designate nucleotide positions which are degenerated. "R" stands for A or G; "Y" stands for C or T; "M" stands for A or C; "K" stands for G or T; "W" stands for A or T; "H" stands for A, C or T; "S" stands for C or G. "I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.

[a] These sequences are the reverse-complement of the selected primers.

Annex XXI: **Sp cific and ubiquitous primers f r nucleic acid amplification (*recA* sequences).**

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment	
		SEQ ID NO.	Nucleotide position
	Universal primers (*recA*)		
919	5'-GGI CCI GAR TCI TMI GGI AAR AC	918[a]	437-459
920[b]	5'-TCI CCV ATI TCI CCI TCI AIY TC	918[a]	701-723
921	5'-TIY RTI GAY GCI GAR CAI GC	918[a]	515-534
922[b]	5'-TAR AAY TTI ARI GCI YKI CCI CC	918[a]	872-894
	Sequencing primers (*recA*)		
1605	5'-ATY ATY GAA RTI TAY GCI CC	1704[a]	220-239
1606	5'-CCR AAC ATI AYI CCI ACT TTT TC	1704[a]	628-650
	Universal primers (*rad51*)		
935	5'-GGI AAR WSI CAR YTI TGY CAY AC	939[a]	568-590
936[b]	5'-TCI SIY TCI GGI ARR CAI GG	939[a]	1126-1145
	Universal primers (*dmc1*)		
937	5'-ATI ACI GAR GYI TTY GGI GAR TT	940[a]	1038-1060
938[b]	5'-CYI GTI GYI SWI GCR TGI GC	940[a]	1554-1573

a Sequences from databases.

b These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex XXII: Specific and ubiquitous primers for nucleic acid amplification (*speA* sequences).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment	
		SEQ ID NO.	Nucleotide position
Bacterial species: ***Streptococcus pyogenes***			
994	5'-TGG ACT AAC AAT CTC GCA AGA GG	993[a]	60-82
995[b]	5'-ACA TTC TCG TGA GTA ACA GGG T	993[a]	173-194
996	5'-ACA AAT CAT GAA GGG AAT CAT TTA G	993[a]	400-424
997[b]	5'-CTA ATT CTT GAG CAG TTA CCA TT	993[a]	504-526
998	5'-GGA GGG GTA ACA AAT CAT GAA GG	993[a]	391-413
997[b]	5'-CTA ATT CTT GAG CAG TTA CCA TT	993[a]	504-526

[a] Sequence from databases.
[b] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex XXIII: First strategy for the selection of *Streptococcus pyogenes*-specific amplification primers from *speA* sequences.

	Accession #	57 ... 85 170 ... 197	SEQ ID NO.:
speA	X61573	CCTT GGgCTAACAA cCTCaCAAGA aGTAT...GTGAtCCT.GT cgtTCAtGAG AATGTAAA	-
speA	AF029051	~~~~ GGgCTAACAA cCTCaCAAGA aGTAT...GTGAtCCT.GT cgtTCAtGAG AATGTAAA	-
speA	X61571	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	-
speA	X61570	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	-
speA	X61568	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	-
speA	X61569	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	-
speA	X61572	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	-
speA	X61560	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	-
speA	U40453	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	993
speA	X61554	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	-
speA	X61557	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	-
speA	X61559	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	-
speA	X61558	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	-
speA	X61556	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	-
speA	X61555	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	-
speA	X61560	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	-
speA	X61561	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	-
speA	X61566	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	-
speA	X61567	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	-
speA	X61562	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	-
speA	X61563	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	-
speA	X61564	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	-
speA	X61565	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	-
speA	AF055698	~~~~ GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	-
speA	X03929[a]	TCTT GGACTAACAA TCTtGCcAaA aGGTA...GTGACCCTGGT TACTCACGAG AATGTGAA	-
Selected sequence for species-specific primer		T GGACTAACAA TCTCGCAAGA GG	994
Selected sequence for species-specific primer[b]		ACCCT.GT TACTCACGAG AATGT	995

The sequence numbering refers to the *Streptococcus pyogenes speA* gene fragment (SEQ ID NO. 993). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. "~" indicate incomplet sequence data. Dots indicate gaps in the sequences displayed.

[a] The extra G nucleotide introducing a gap in the sequence is probably a sequencing error.

[b] This sequence is the reverse-complement of the selected primer.

Ann x XXIV: Second strategy for the selection of *Streptococcus pyogenes*-sp cific amplification primers from *speA* sequences.

	Accession #	388 ... 427 501 ... 529	SEQ ID NO.:
speA	X61573	TA TGGAGGGGTA ACAAATCATG AAGGGAATCA TTTAGAAA...AAAAATGGT AACTGCTCAA GAATTAGACT	-
speA	AF029051	TA TGGAGGGGTA ACAAATCATG AAGGGAATCA TTTAGAAA...AAAAATGGT AACTGCTCAA GAATTAGACT	-
speA	X61571	TA CGGAGGGGTA ACAAATCATG AAGGGAATCA TTTAGAAA...AAAAATGGT AACTGCTCAA GAATTAGACT	-
speA	X61570	TA CGGAGGGGTA ACAAATCATG AAGGGAATCA TTTAGAAA...AAAAATGGT AACTGCTCAA GAATTAGACT	-
speA	X61568	TA CGGAGGGGTA ACAAATCATG AAGGGAATCA TTTAGAAA...AAAAATGGT AACTGCTCAA GAATTAGACT	-
speA	X61569	TA CGGAGGGGTA ACAAATCATG AAGGGAATCA TTTAGAAA...AAAAATGGT AACTGCTCAA GAATTAGACT	-
speA	X61572	TA CGGAGGGGTA ACAAATCATG AAGGGAATCA TTTAGAAA...AAAAATGGT AACTGCTCAA GAATTAGACT	-
speA	X61560	TA CGGAGGGGTA ACAAATCATG AAGGGAATCA TTTAGAAA...AAAAATGGT AACTGCTCAA GAATTAGACT	993
speA	U40453	TA CGGAGGGGTA ACAAATCATG AAGGGAATCA TTTAGAAA...AAAAATGGT AACTGCTCAA GAATTAGACT	-
speA	X61554	TA CGGAGGGGTA ACAAATCATG AAGGGAATCA TTTAGAAA...AAAAATGGT AACTGCTCAA GAATTAGACT	-
speA	X61557	TA CGGAGGGGTA ACAAATCATG AAGGGAATCA TTTAGAAA...AAAAATGGT AACTGCTCAA GAATTAGACT	-
speA	X61559	TA CGGAGGGGTA ACAAATCATG AAGGGAATCA TTTAGAAA...AAAAATGGT AACTGCTCAA GAATTAGACT	-
speA	X61558	TA CGGAGGGGTA ACAAATCATG AAGGGAATCA TTTAGAAA...AAAAATGGT AACTGCTCAA GAATTAGACT	-
speA	X61556	TA CGGAGGGGTA ACAAATCATG AAGGGAATCA TTTAGAAA...AAAAATGGT AACTGCTCAA GAATTAGACT	-
speA	X61555	TA CGGAGGGGTA ACAAATCATG AAGGGAATCA TTTAGAAA...AAAAATGGT AACTGCTCAA GAATTAGACT	-
speA	X61560	TA CGGAGGGGTA ACAAATCATG AAGGGAATCA TTTAGAAA...AAAAATGGT AACTGCTCAA GAATTAGACT	-
speA	X61561	TA CGGAGGGGTA ACAAATCATG AAGGGAATCA TTTAGAAA...AAAAATGGT AACTGCTCAA GAATTAGACT	-
speA	X61566	TA CGGAGGGGTA ACAAATCATG AAGGGAATCA TTTAGAAA...AAAAATGGT AACTGCTCAA GAATTAGACT	-
speA	X61567	TA CGGAGGGGTA ACAAATCATG AAGGGAATCA TTTAGAAA...AAAAATGGT AACTGCTCAA GAATTAGACT	-
speA	X61562	TA CGGAGGGGTA ACAAATCATG AAGGGAATCA TTTAGAAA...AAAAATGGT AACTGCTCAA GAATTAGACT	-
speA	X61563	TA CGGAGGGGTA ACAAATCATG AAGGGAATCA TTTAGAAA...AAAAATGGT AACTGCTCAA GAATTAGACT	-
speA	X61564	TA CGGAGGGGTA ACAAATCATG AAGGGAATCA TTTAGAAA...AAAAATGGT AACTGCTCAA GAATTAGACT	-
speA	X61565	TA CGGAGGGGTA ACAAATCATG AAGGGAATCA TTTAGAAA...AAAAATGGT AACTGCTCAA GAATTAGACT	-
speA	AF055698	TA CGGAGGGGTA ACAAATCATG AAGGGAATCA TTTAGAAA...AAAAATGGT AACTGCTCAA GAATTAGACT	-
speA	X03929	TA CGGAGGGGTA ACAAATCATG AAGGGAATCA TTTAGAAA...AAAAATGGT AACTGCTCAA GAATTAG.CT	
Selected sequences for species-specific primers		GGAGGGGTA ACAAATCATG AAGG	998
		ACAAATCATG AAGGGAATCA TTTAG	996
Selected sequence for species-specific primer[a]		AATGGT AACTGCTCAA GAATTAG	997

The sequence numbering refers to the *Streptococcus pyogenes speA* gene fragment (SEQ ID NO. 993). Dots indicate gaps in the sequences displayed.

[a] This sequence is the reverse-complement of the selected primer.

Annex XXV: Strategy for the selection of *Streptococcus pyogenes*-specific amplification primers from *tuf* sequences.

	140 ... 186 619 ... 647	SEQ ID NO.:
S. anginosus	A AGTTGACtTg GTTGACGAtG AAGAaTTGCT TGAATTgGTT GAaATG...CC AgGTTCAATt cAtCCACACA CTAAATT	211
S. bovis	A AGTTGACCTT GTTGATGACG AAGAaTTGCT TGAATTgGTT GAaATG...CC AgGTTCAATC cACCCACACA CTAAATT	212
S. dysgalactiae	A AATTGACCTT GTTGAcGAtG AAGAaTTGCT TGAATTgGTT GAaATG...CC AgGTTCAATC AACCCACACA CTAAATT	217
S. pyogenes	A AGTTGACCTT GTTGATGACG AAGAGTTGCT TGAATTAGTT GAGATG...CC AAGTTCAATC AACCCACACA CTAAATT	1002
S. agalactiae	A AGTTGACCTT GTTGATGAtG AAGAaTTGCT TGAATTgGTT GAaATG...CC AgGTTCAATC AACCCACACA CTAAATT	144[a]
S. oralis	A AATTGACtTg GTAGAcGACG AAGAaTTGCT TGAATTgGTT GAaATG...CC AgGTTCAATC AACCCACACA CTAAATT	985
S. pneumoniae	A AGTTGACtTg GTTGAcGACG AAGAaTTGCT TGAATTgGTT GAaATG...CC AgGTTCAATC AACCCACACA CTAAATT	145[a]
S. cristatus	A GATCGACtTg GTTGATGACG AAGAaTTGCT TGAATTgGTT GAaATG...CC AgGTTCAATC AACCCACACA CTAAATT	215
S. mitis	A GATCGACtTg GTTGATGACG AAGAaTTGCT TGAATTgGTT GAaATG...CC AgGTTCAATC AACCCACACA CTAAATT	982
S. gordonii	A AGTTGACtTg GTTGAcGAtG AAGAaTTGCT TGAGTTgGTT GAaATG...CC AgGTTCAATC AACCCACACA CTAAATT	200
S. sanguinis	A AGTTGACtTg GTTGAcGAtG AAGAaTTGCT TGAATTgGTT GAaATG...CC AgGTTCAATC AACCCACACA CTAAATT	227
S. parasanguinis	A AGTTGACtTg GTTGATGAtG AAGAaTTGCT TGAATTgGTT GAaATG...CC AgGTTCAATC AACCCACACA CTAAATT	225
S. salivarius	A AGTTGACtTg GTTGAcGAtG AAGAaTTGCT TGAATTgGTT GAaATG...CC TgGTTCAATC AACCCACACA CTAAATT	146[a]
S. vestibularis	A AGTTGACtTg GTTGAcGAtG AAGAaTTGCT TGAATTgGTT GAaATG.. CC TgGTTCAATC AACCCACACA CTAAATT	231
S. suis	A AGTTGACtTg GTTGAcGAtG AAGAaTTGCT TGAgTTgGTT GAaATG...CC AgGTTCtATC AACCCACACA CTAAATT	229
S. mutans	A AGTTGACtTg GTTGAcGAtG AAGAaTTGCT TGAATTgGTT GAaATG...CC AgGTTCAATt cACCCACAcA CTAAATT	224
S. ratti	A AGTTGAttTg GTTGAcGAtG AAGAaTTGCT TGAATTgGTT GAaATG...CC AgGTTCAATt cAtCCgCAcA CTAAATT	226
S. macacae	A GGTTGACtTg GTTGATGAtG AAGAaTTGCT TGAATTgGTT GAaATG...CC AgGATCAATt cAtCCACAcA CTAAATT	222
S. cricetus	A AGTTGACtTa GTTGATGAtG AAGAaTTGCT TGAATTgGTT GAaATG...CC TgGTTCAATC cAtCCACACA CTAAATT	214
E. faecalis	A GGTTGACtTg GTTGAcGAtG AAGAaTTGCT TGAATTgGTT GAaATG...CC AgcTaCAATC ActCCACACA CaAAATT	607
S. aureus	A AATgGAtaTg GTTGATGACG AAGAaTTatT aGAATTAGTa GAaATG...CC TgGTTCAATt AcaCCACACA CTgAATT	176
B. cereus	A AGTTGACaTg GTTGAcGAtG AAGAaTTatT aGAATTAGTa GAaATG...CC CgGTTCtgTa AAagCtCACg CTAAATT	7
E. coli	A ATgcGACaTg GTaGATGACG AAGAaTTatT aGAATTAGTa GAaATG...AG GgGCaCcATC AAgCCgCACA CcAAGTT	78
Selected sequences for species-specific primers	TTGACCTT GTTGATGACG AAGAG	999
	AAGAGTTGCT TGAATTAGTT GAG	1001
Selected sequence for species-specific primer[b]	AGTTCAATC AACCCACACA CTAA	1000

The sequence numbering refers to the *Streptococcus pyogenes tuf* gene fragment (SEQ ID NO. 1002). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. Dots indicate gaps in the sequences displayed.

[a] The SEQ ID NO. refers to previous patent publication WO98/20157.

[b] This sequence is the reverse-complement of the selected primer.

Ann x XXVI: Strategy for the selection stx_1-specific amplification primers and hybridization probe.

	Accession #	230			263	343			375	391			421	SEQ ID NO.:
stx₁	M19473a	TTGATGTC	AGAGGGATAG	ATCCAGAGGA	AGGGCG...	TATCG	CTTTGCTGAT	TTTTCACATG	TTACCTTT...	GTTACAT	TGTCTGGTGA	CAGTAGCTAT	ACCA	-
stx₁	M16625	TTGATGTC	AGAGGGATAG	ATCCAGAGGA	AGGGCG...	TATCG	CTTTGCTGAT	TTTTCACATG	TTACCTTT...	GTTACAT	TGTCTGGTGA	CAGTAGCTAT	ACCA	-
stx₁	M17358	TTGATGTC	AGAGGGATAG	ATCCAGAGGA	AGGGCG...	TATCG	CTTTGCTGAT	TTTTCACATG	TTACCTTT...	GTTACAT	TGTCTaGTGA	CAGTAGCTAT	ACCA	-
stx₁	Z36900	TTGATGTC	AGAGGGATAG	ATCCAGAGGA	AGGGCG...	TATCG	CTTTGCTGAT	TTTTCACATG	TTACCTTT...	GTTACAT	TGTCTGGTGA	CAGTAGCTAT	ACCA	-
stx₁	L04539	TTGATGTC	AGAGGGATAG	ATCCAGAGGA	AGGGCG...	TATCG	CTTTGCTGAT	TTTTCACATG	TTACCTTT...	GTTACAT	TGTCTGGTGA	CAGTAGCTAT	ACCA	-
stx₁	M19437	TTGATGTC	AGAGGGATAG	ATCCAGAGGA	AGGGCG...	TATCG	CTTTGCTGAT	TTTTCACATG	TTACCTTT...	GTTACAT	TGTCTGGTGA	CAGTAGCTAT	ACCA	-
stx₁	M24352	TTGATGTC	AGAGGGATAG	ATCCAGAGGA	AGGGCG...	TATCG	CTTTGCTGAT	TTTTCACATG	TTACCTTT...	GTTACAT	TGTCTGGTGA	CAGTAGCTAT	ACCA	-
stx₁	X07903	TTGATGTC	AGAGGGATAG	ATCCAGAGGA	AGGGCG...	TATCG	CTTTGCTGAT	TTTTCACATG	TTACCTTT...	GTTACAT	TGTCTGGTGA	CAGTAGCTAT	ACCA	-
stx₁	Z36899	TTGATGTC	AGAGGGATAG	ATCCAGAGGA	AGGGCG...	TATCG	CTTTGCTGAT	TTTTCACATG	TTACCTTT...	GTTACAT	TGTCTGGTGA	CAGTAGCTAT	ACCA	-
stx₁	Z36901	TTGATGTC	AGAGGGATAG	ATCCAGAGGA	AGGGCG...	TATCG	CTTTGCTGAT	TTTTCACATG	TTACCTTT...	GTTACAT	TGTCTGGTGA	CAGTAGCTAT	ACCA	1076
stx₂	X61283	TGGATaTa	cGAGGGcTtG	ATgtctAtcA	gGcGCG...	TACCG	tTTTtCaGAT	TTTaCACATa	TatCaGTG...	GTTtCca	TGaCaacgGA	CAGcAGtTAT	ACCA	-
stx₂	L11079	TGGATaTa	cGAGGGcTtG	ATgtctAtcA	gGcGCG...	TACCG	tTTTtCaGAT	TTTaCACATa	TatCaGTG...	GTTtCca	TGaCaacgGA	CAGcAGtTAT	ACCA	-
stx₂	M21534	TAGgTaTa	cGAGGGcTtG	ATgtttAtcA	gGaGCG...	TACaG	aTTTtCaGAT	TTTgCACATa	TatCaTTG...	ATTtCca	TGaCaacgGA	CAGcAGtTAT	ACCA	-
stx₂	M36727	TAGgTaTa	cGAGGGcTtG	ATgtttAtcA	gGaGCG...	TACaG	aTTTtCaGAT	TTTgCACATa	TatCaTTG...	ATTtCca	TGaCaacgGA	CAGcAGtTAT	ACCA	-
stx₂	X81415	TAGgTaTa	cGAGGGcTtG	ATgtttAtcA	gGaGCG...	TACaG	aTTTtCaGAT	TTTgCACATa	TatCaTTG...	ATTtCca	TGaCaacgGA	CAGcAGtTAT	ACCA	-
stx₂	X81416	TAGgTaTa	cGAGGGcTtG	ATgtttAtcA	gGaGCG...	TACaG	aTTTtCaGAT	TTTgCACATa	TatCaTTG...	ATTtCca	TGaCaacgGA	CAGcAGtTAT	ACCA	-
stx₂	X81417	TAGgTaTa	cGAGGGcTtG	ATgtttAtcA	gGaGCG...	TACaG	aTTTtCaGAT	TTTgCACATa	TatCaTTG...	ATTtCca	TGaCaacgGA	CAGcAGtTAT	ACCA	-
stx₂	X81418	TGGATaTa	cGAGGGcTtG	ATgtctAtcA	gGcGCG...	TACCG	tTTTtCaGAT	TTTaCACATa	TatCaGTG...	GTTtCca	TGaCaacgGA	CAGcAGtTAT	ACCA	-
stx₂	E03962	TGGATaTa	cGAGGGcTtG	ATgtctAtcA	gGcGCG...	TACCG	tTTTtCaGAT	TTTaCACATa	TatCaGTG...	GTTtCca	TGaCaacgGA	CAGcAGtTAT	ACCA	-
stx₂	E03959	TGGATaTa	cGAGGGcTtG	ATgtctAtcA	gGcGCG...	TACCG	tTTTtCaGAT	TTTaCACATa	TatCaGTG...	GTTtCca	TGaCaacgGA	CAGcAGtTAT	ACCA	-
stx₂	X07865	TGGATaTa	cGAGGGcTtG	ATgtctAtcA	gGcGCG...	TACCG	tTTTtCaGAT	TTTaCACATa	TatCaGTG...	GTTtCca	TGaCaacgGA	CAGcAGtTAT	ACCA	-
stx₂	Y10775	TGGATaTa	cGAGGGcTtG	ATgtctAtcA	gGcGCG...	TACCG	tTTTtCaGAT	TTTaCACATa	TatCaGTG...	GTTtCca	TGaCaacgGA	CAGcAGtTAT	ACCA	-
stx₂	Z37725	TGGATaTa	cGAGGGcTtG	ATgtctAtcA	gGcGCG...	TACCG	tTTTtCaGAT	TTTaCACATa	TatCaGTG...	GTTtCca	TGaCaacgGA	CAGcAGtTAT	ACCA	1077
stx₂	Z50754	TGGATaTa	cGAGGGcTtG	ATgtctAtcA	gGcGCG...	TACCG	tTTTtCaGAT	TTTaCACATa	TatCaGTG...	GTTtCca	TGaCaacgGA	CAGcAGtTAT	ACCA	-
stx₂	X67514	TGGATaTa	cGAGGGcTtG	ATgtctAtcA	gGcGCG...	TACCG	tTTTtCaGAT	TTTaCACATa	TatCaGTG...	GTTtCca	TGaCaacgGA	CAGcAGtTAT	ACCA	-
stx₂	L11078	TGGATaTa	cGAGGGcTtG	ATgtctAtcA	gGcGCG...	TACCG	tTTTtCaGAT	TTTaCACATa	TatCaGTG...	GTTtCca	TGaCaacgGA	CAGcAGtTAT	ACCA	-
stx₂	X65949	TGGATaTa	cGAGGGcTtG	ATgtctAtcA	gGcGCG...	TACCG	tTTTtCaGAT	TTTaCACATa	TatCaGTG...	GTTtCca	TGaCaacgGA	CAGcAGtTAT	ACCA	-
stx₂	AF043627	TGGATaTa	cGAGGGcTtG	ATgtctAtcA	gGcGCG...	TACaG	aTTTtCaGAT	TTTgCACATa	TatCaGTG...	GTTtCca	TGaCaacgGA	CAGcAGtTAT	ACCA	-
Selected sequence for amplification primer		ATGTC	AGAGGGATAG	ATCCAGAGGA	AGG									1081
Selected sequence for hybridization probe						CG	CTTTGCTGAT	TTTTCACATG	TTACC					1084
Selected sequence for amplification primer[a]										ACAT	TGTCTGGTGA	CAGTAGCTAT	A	1080

The sequence numbering refers to the *Escherichia coli* stx_1 gene fragment (SEQ ID NO. 1076). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. Dots indicate gaps in the sequences displayed.

[a] This sequence is the reverse-complement of the selected primer.

Ann x XXVII: Strategy for the selection of stx_2-specific amplification primers and hybridization probe.

	Accession #	543			570 614			641 684			708	SEQ ID NO.:
stx_1	M19473	AGCga	TgtTaCGgTT	TGTtACTGTG	ACA...CAAC	ACTGgaTGAt	ctcAgTGggC	gTtcTTA...A	AGGtTgAGtA	gCGTcCTgCC	tGAC	-
stx_1	M16625	AGCga	TgtTaCGgTT	TGTtACTGTG	ACA...CAAC	ACTGgaTGAt	ctcAgTGggC	gTtcTTA...A	AGGtTgAGtA	gCGTcCTgCC	tGAC	-
stx_1	M17358	AGCga	TgtTaCGgTT	TGTtACTGTG	ACA...CAAC	ACTGgaTGAt	ctcAgTGggC	gTtcTTA...A	AGGtTgAGtA	gCGTcCTgCC	tGAC	-
stx_1	Z36900	AGCga	TgtTaCGgTT	TGTtACTGTG	ACA...CAAC	ACTGgaTGAt	ctcAgTGggC	gTtcTTA...A	AGGtTgAGtA	gTGTcCTgCC	tGAT	-
stx_1	L04539	AGCga	TgtTaCGgTT	TGTtACTGTG	ACA...CAAC	ACTGgaTGAt	ctcAgTGggC	gTtcTTA...A	AGGtTgAGtA	gTGTcCTgCC	tGAT	-
stx_1	M19437	AGCga	TgtTaCGgTT	TGTtACTGTG	ACA...CAAC	ACTGgaTGAt	ctcAgTGggC	gTtcTTA...A	AGGtTgAGtA	gTGTcCTgCC	tGAC	-
stx_1	M24352	AGCga	TgtTaCGgTT	TGTtACTGTG	ACA...CAAC	ACTGgaTGAt	ctcAgTGggC	gTtcTTA...A	AGGtTgAGtA	gTGTcCTgCC	tGAC	-
stx_1	X07903	AGCga	TgtTaCGgTT	TGTtACTGTG	ACA...CAAC	ACTGgaTGAt	ctcAgTGggC	gTtcTTA...A	AGGtTgAGtA	gTGTcCTgCC	tGAC	-
stx_1	Z36899	AGCga	TgtTaCGgTT	TGTtACTGTG	ACA...CAAC	ACTGgaTGAt	ctcAgTGggC	gTtcTTA...A	AGGtTgAGtA	gTGTcCTgCC	tGAC	-
stx_1	Z36901	AGCga	TgtTaCGgTT	TGTtACTGTG	ACA...CAAC	ACTtgaTGAt	ctcAgTGggC	gTtcTTA...A	AGGtTgAGtA	gTGTcCTgCC	tGAC	1076
stx_2	X61283	AGCAG	TTCTGCGTTT	TGTCACTGTC	ACA...AGGC	ACTGTCTGA.	..AACTGCTC	CTGTGTA...G	CGAATCAGCA	ATGTGCTTCC	GGAG	-
stx_2	L11079	AGCAG	TTCTGCGTTT	TGTCACTGTC	ACA...AGGC	ACTGTCTGA.	..AACTGCTC	CTGTGTA...G	CGAATCAGCA	ATGTGCTTCC	GGAG	-
stx_2	M21534	AGCAG	TTCTGCGTTT	TGTCACTGTC	ACA...TGGC	ACTGTCTGA.	..AACTGCTC	CTGTTTA...G	AGAATCAGCA	ATGTGCTTCC	GGAG	-
stx_2	M36727	AGCAG	TTCTGCGTTT	TGTCACTGTC	ACA...TGGC	ACTGTCTGA.	..AACTGCTC	CTGTTTA...G	AGAATCAGCA	ATGTGCTTCC	GGAG	-
stx_2	U72191	AGCAG	TTCTGCGTTT	TGTCACTGTC	ACA...TGGC	ACTGTCTGA.	..AACTGCTC	CTGTTTA...G	AGAATCAGCA	ATGTGCTTCC	GGAG	-
stx_2	X81415	AGCAG	TTCTGCGTTT	TGTCACTGTC	ACA...TGGC	ACTGTCTGA.	..AACTGCTC	CTGTTTA...G	AGAATCAGCA	ATGTGCTTCC	GGAG	-
stx_2	X81416	AGCAG	TTCTGCGTTT	TGTCACTGTC	ACA...TGGC	ACTGTCTGA.	..AACTGCTC	CTGTTTA...G	AGAATCAGCA	ATGTGCTTCC	GGAG	-
stx_2	X81417	AGCAG	TTCTGCGTTT	TGTCACTGTC	ACA...TGGC	ACTGTCTGA.	..AACTGCTC	CTGTTTA...G	AGAATCAGCA	ATGTGCTTCC	GGAG	-
stx_2	X81418	AGCAG	TTCTGCGTTT	TGTCACTGTC	ACA...TGGC	ACTGTCTGA.	..AACTGCTC	CTGTTTA...G	AGAATCAGCA	ATGTGCTTCC	GGAG	-
stx_2	E03962	AGCAG	TTCTGCGTTT	TGTCACTGTC	ACA...AGGC	ACTGTCTGA.	..AACTGCTC	CTGTGTA...G	CGAATCAGCA	ATGTGCTTCC	GGAG	-
stx_2	E03959	AGCAG	TTCTGCGTTT	TGTCACTGTC	ACA...AGGC	ACTGTCTGA.	..AACTGCTC	CTGTGTA...G	CGAATCAGCA	ATGTGCTTCC	GGAG	-
stx_2	X07865	AGCAG	TTCTGCGTTT	TGTCACTGTC	ACA...AGGC	ACTGTCTGA.	..AACTGCTC	CTGTGTA...G	CGAATCAGCA	ATGTGCTTCC	GGAG	-
stx_2	Y10775	AGCAG	TTCTGCGTTT	TGTCACTGTC	ACA...AGGC	ACTGTCTGA.	..AACTGCTC	CTGTGTA...G	CGAATCAGCA	ATGTGCTTCC	GGAG	-
stx_2	Z37725	AGCAG	TTCTGCGTTT	TGTCACTGTC	ACA...AGGC	ACTGTCTGA.	..AACTGCTC	CTGTGTA...G	CGAATCAGCA	ATGTGCTTCC	GGAG	1077
stx_2	Z50754	AGCAG	TTCTGCGTTT	TGTCACTGTC	ACA...AGGC	ACTGTCTGA.	..AACTGCTC	CTGTGTA...G	CGAATCAGCA	ATGTGCTTCC	GGAG	-
stx_2	X67514	AGCAG	TTCTGCGTTT	TGTCACTGTC	ACA...AGGC	ACTGTCTGA.	..AACTGCTC	CTGTGTA...G	CGAATCAGCA	ATGTGCTTCC	GGAG	-
stx_2	L11078	AGCAG	TTCTGCGTTT	TGTCACTGTC	ACA...AGGC	ACTGTCTGA.	..AACTGCTC	CTGTGTA...G	AGAATCAGCA	ATGTGCTTCC	GGAG	-
stx_2	X65949	AGCAG	TTCTGCGTTT	TGTCACTGTC	ACA...AGGC	ACTGTCTGA.	..AACTGCTC	CTGTGTA...G	AGAATCAGCA	ATGTGCTTCC	GGAG	-
stx_2	AF043627	AGCAG	TTCTGCGTTT	TGTCACTGTC	ACA...TGGC	ACTGTCTGA.	..AACTGCTC	CTGTTTA...G	AGAATCAGCA	ATGTGCTTCC	GGAG	-
Selected sequence for amplification primer		AG	TTCTGCGTTT	TGTCACTGTC								1078
Selected sequence for hybridization probe						C ACTGTCTGA.	..AACTGCTC	CTGT				1085
Selected sequence for amplification primer[a]									AATCAGCA	ATGTGCTTCC	G	1079

The sequence numbering refers to the *Escherichia coli* stx_2 gene fragment (SEQ ID NO. 1077). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. Dots indicate gaps in the sequences displayed.

[a] This sequence is the reverse-complement of the selected primer.

Ann x XXVIII: Strategy for the selection of *vanA*-specific amplification primers from *van* sequences.

	Accession #	926 ... 952 1230 ... 1255	SEQ ID NO.:
vanA	X56895	GTCAAT AGCGCGGACG AATTGGACTA C...GT AGAGGTCTAG CCCGTGTGGA TATG	1139
vanA	M97297	GTCAAT AGCGCGGACG AATTGGACTA C...GT AGAGGTCTAG CCCGTGTGGA TATG	1141
vanA	-	GTCAAT AGCGCGGACG AATTGGACTA C...GT AGAGGTCTAG CCCGTGTGGA TATG	1051
vanA	-	GTCAAT AGCGCGGACG AATTGGACTA C...GT AGAGGTCTAG CCCGTGTGGA TATG	1052
vanA	-	GTCAAT AGCGCGGACG AATTGGACTA C...GT AGAGGTCTAG CCCGTGTGGA TATG	1053
vanA	-	GTCAAT AGCGCGGACG AATTGGACTA C...GT AGAGGTCTAG CCCGTGTGGA TATG	1054
vanA	-	GTCAAT AGCGCGGACG AATTGGACTA C...GT AGAGGTCTAG CCCGTGTGGA TATG	1055
vanA	-	GTCAAT AGCGCGGACG AATTGGACTA C...GT AGAGGTCTAG CCCGTGTGGA TATG	1056
vanA	-	GTCAAT AGCGCGGACG AATTGGACTA C...GT AGAGGTCTAG CCCGTGTGGA TATG	1057
vanA	-	GTCAAT AGCGCGGACG AATTGGACTA C...GT AGAGGTCTAG CCCGTGTGGA TATG	1049
vanA	-	GTCAAT AGCGCGGACG AATTGGACTA C...GT AGAGGTCTAG CCCGTGTGGA TATG	1050
vanB	U94526	GTAAAc gGtaCGGAaG AAcTtaACGC T...GC AGAGGgCTtG CCCGTGTtGA TCTT	1117
vanB	U94527	GTAAAc AGtaCGGAaG AAcTaaACGC T...GC AGAGGgCTtG CtCGTGTtGA TCTT	-
vanB	U94528	GTAAAc gGtaCGGAaG AAcTtaACGC T...GC AGAGGgCTtG CCCGTGTtGA TCTT	-
vanB	U94529	GTAAAc gGtaCGGAaG AAcTtaACGC T...GC AGAGGgCTtG CCCGTGTtGA TCTT	-
vanB	U94530	GTAAAc gGtaCGGAaG AAcTtaACGC T...GC AGAGGgCTtG CCCGTGTtGA TCTT	-
vanB	Z83305	GTAAAc gGtaCGGAaG AAcTtaACGC T...GC AGAGGgCTtG CCCGTGTtGA TCTT	-
vanB	U81452	GTAAAc gGtaCGGAaG AAcTtaACGC T...GC AGAGGgCTtG CCCGTGTtGA TCTT	-
vanB	U35369	GTAAAc AGtaCGGAaG AAcTaaACGC T...GC AGAGGgCTtG CtCGTGTtGA TCTT	-
vanB	U72704	GTAAAc gGtaCGGAaG AAcTtaACGC T...GC AGAGGgCTtG CCCGTGTtGA TCTT	-
vanB	L06138	GTAAAc AGtaCGGAaG AAcTaaACGC T...GC AGAGGgCTtG CtCGTGTtGA TCTT	-
vanB	L15304	GTAAAc gGtaCGGAaG AAcTtaACGC T...GC AGAGGgCTtG CCCGTGTtGA TCTT	-
vanB	U00456	GTAAAc AGtaCGGAaG AAcTaaACGC T...GC AGAGGgCTtG CtCGTGTtGA TCTT	-
vanD	AF130997	GTAtgc AagGCaGAaG AAcTGcAgGC A...GC AGAGGatTgG CCCGcaTtGA cCTG	-
vanE	AF136925	GTAgAa caaaaaagtG AtTTatAtAA A...GC AaAGGatTAG CgaGaaTcGA cTTT	-
Selected sequence for amplification primer		AAT AGCGCGGACG AATTGGAC	1090
Selected sequence for amplification primer[a]		GAGGTCTAG CCCGTGTGGA T	1089

The sequence numbering refers to the *Enterococcus faecium vanA* gene fragment (SEQ ID NO. 1139). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. Dots indicate gaps in the sequences displayed.

[a] This sequence is the reverse-complement of the above selected primer.

Annex XXIX: Strategy for the selection of *vanB*-specific amplification primers from *van* sequences.

	Accession #	470			495 608			633	SEQ ID NO.:
vanA	X56895	A	**CGCaATtGAA**	**tCgGCAaGAC**	**AATAT**...ACG	**GaATCTTtCG**	**tATtCATCAG**	GAA	1139
vanA	M97297	A	**CGCaATtGAA**	**tCgGCAaGAC**	**AATAT**...ACG	**GaATCTTtCG**	**tATtCATCAG**	GAA	1141
vanA	-	A	**CGCaATtGAA**	**tCgGCAaGAC**	**AATAT**...ACG	**GaATCTTtCG**	**tATtCATCAG**	GAA	1051
vanA	-	A	**CGCaATtGAA**	**tCgGCAaGAC**	**AATAT**...ACG	**GaATCTTtCG**	**tATtCATCAG**	GAA	1052
vanA	-	A	**CGCaATtGAA**	**tCgGCAaGAC**	**AATAT**...ACG	**GaATCTTtCG**	**tATtCATCAG**	GAA	1053
vanA	-	A	**CGCaATtGAA**	**tCgGCAaGAC**	**AATAT**...ACG	**GaATCTTtCG**	**tATtCATCAG**	GAA	1054
vanA	-	A	**CGCaATtGAA**	**tCgGCAaGAC**	**AATAT**...ACG	**GaATCTTtCG**	**tATtCATCAG**	GAA	1055
vanA	-	A	**CGCaATtGAA**	**tCgGCAaGAC**	**AATAT**...ACG	**GaATCTTtCG**	**tATtCATCAG**	GAA	1056
vanA	-	A	**CGCaATtGAA**	**tCgGCAaGAC**	**AATAT**...ACG	**GaATCTTtCG**	**tATtCATCAG**	GAA	1057
vanA	-	A	**CGCaATtGAA**	**tCgGCAaGAC**	**AATAT**...ACG	**GaATCTTtCG**	**tATtCATCAG**	GAA	1049
vanA	-	A	**CGCaATtGAA**	**tCgGCAaGAC**	**AATAT**...ACG	**GaATCTTtCG**	**tATtCATCAG**	GAA	1050
vanB	U94526	C	**TGCGATAGAA**	**GCgGCAGGAC**	**AATAT**...ACG	**GTATCTTCCG**	**CATCCATCAG**	GAA	1117
vanB	U94527	C	**TGCGATAGAA**	**GCAGCAGGAC**	**AATAT**...ACG	**GTATCTTCCG**	**CATCCATCAG**	GAA	-
vanB	U94528	C	**TGCGATAGAA**	**GCgGCAGGAC**	**AATAT**...ACG	**GTATCTTCCG**	**CATCCATCAG**	GAA	-
vanB	U94529	C	**TGCGATAGAA**	**GCgGCAGGAC**	**AATAT**...ACG	**GTATCTTCCG**	**CATCCATCAG**	GAA	-
vanB	U94530	C	**TGCGATAGAA**	**GCgGCAGGAC**	**AATAT**...ACG	**GTATCTTCCG**	**CATCCATCAG**	GAA	-
vanB	Z83305	C	**TGCGATAGAA**	**GCgGCAGGAC**	**AATAT**...ACG	**GTATCTTCCG**	**CATCCATCAG**	GAA	-
vanB	U81452	C	**TGCGATAGAA**	**GCgGCAGGAC**	**AATAT**...ACG	**GTATCTTCCG**	**CATCCATCAG**	GAA	-
vanB	U35369	C	**TGCGATAGAA**	**GCAGCAGGAC**	**AATAT**...ACG	**GTATCTTCCG**	**CATCCATCAG**	GAA	-
vanB	U72704	C	**TGCGATAGAA**	**GCgGCAGGAC**	**AATAT**...ATG	**GTATCTTCCG**	**CATCCATCAG**	GAA	-
vanB	L06138	C	**TGCGATAGAA**	**GCAGCAGGAC**	**AATAT**...ACG	**GTATCTTCCG**	**CATCCATCAG**	GAA	-
vanB	L15304	C	**TGCGATAGAA**	**GCgGCAGGAC**	**AATAT**...ACG	**GTATCTTCCG**	**CATCCATCAG**	GAA	-
vanB	U00456	C	**TGCGATAGAA**	**GCAGCAGGAC**	**AATAT**...ACG	**GTATCTTCCG**	**CATCCATCAG**	GAA	-
vanD	AF130997	C	**AGCaATcGAA**	**GaAGCAaGAa**	**AATAT**...ACG	**GctTtTTtaa**	**gATtCATCAG**	GAA	-
vanE	AF136925	A	**AGCaATAGAc**	**GaAGCttcAa**	**AATAT**...ATG	**GctTtTTCga**	**CtatgAagAG**	AAA	-
Selected sequence for amplification primer			**CGATAGAA**	**GCAGCAGGAC**	**AA**				1095
Selected sequence for amplification primer[a]						**GTATCTTCCG**	**CATCCATCAG**		1096

The sequence numbering refers to the *Enterococcus faecium vanB* gene fragment (SEQ ID NO. 1117). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. Dots indicate gaps in the sequences displayed.

[a] This sequence is the reverse-complement of the above *vanB* sequence.

Annex XXX: Strategy for the selection of *vanC*-specific amplification primers from *vanC* sequences.

	Accession #	929	957 1064	1092	SEQ ID NO.:
vanC1	-	GT CGACGGTTTT TTTGATTTTG AAGAGAA...	...ACGGGTC TGGCTCGAAT CGATTTTTTC	GT	1058
vanC1	-	GT CGACGGTTTT TTTGATTTTG AAGAGAA...	...ACGGGTC TGGCTCGAAT CGATTTTTTC	GT	1059
vanC1	M75132	GT CGACGGTTTT TTTGATTTTG AAGAGAA...	...ACGGGTC TGGCTCGAAT CGATTTTTTC	GT	1138
vanC2	-	GT AGACGGCTTT TTCGATTTTG AAGAAAA...	...AAAGGTC TTGCTCGCAT CGACTTTTTT	GT	1060
vanC2	-	GT AGACGGCTTT TTCGATTTTG AAGAAAA...	...AAAGGTC TTGCTCGCAT CGACTTTTTT	GT	1061
vanC2	-	GT AGACGGCTTT TTCGATTTTG AAGAAAA...	...AAAGGTC TTGCTCGCAT CGACTTTTTT	GT	1062
vanC2	-	GT AGACGGCTTT TTCGATTTTG AAGAAAA...	...AAAGGTC TTGCTCGCAT CGACTTTTTT	GT	1063
vanC2	L29638	GT AGACGGCTTT TTCGATTTTG AAGAAAA...	...AAAGGTC TTGCTCGCAT CGACTTTTTT	GT	-
vanC2	L29638	GT AGACGGCTTT TTCGATTTTG AAGAAAA...	...AAAGGTC TTGCTCGCAT CGACTTTTTT	GT	-
vanC3	-	GT AGACGGCTTT TTCGATTTTG AAGAAAA...	...AAAGGTC TTGCTCGCAT CGACTTTTTT	GT	1064
vanC3	-	GT AGACGGCTTT TTCGATTTTG AAGAAAA...	...AAAGGTC TTGCTCGCAT CGACTTTTTT	GT	1065
vanC3	-	GT AGACGGCTTT TTCGATTTTG AAGAAAA...	...AAAGGaC TTGCTCGCAT CGACTTTTTT	GT	1066
vanC3	L29639	GT AGACGGCTTT TTCGATTTTG AAGAAAA...	...AAAGGTC TTGCTCGCAT CGACTTTTTT	GT	-
Selected sequence for resistance primer		GACGGYTTT TTYGATTTTG AAGA			1101
Selected sequence for resistance primer[a]			GGTC TKGCTCGMAT CGAYTTTTT		1102

The sequence numbering refers to the *vanC1* gene fragment (SEQ ID NO. 1138). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. Dots indicate gaps in the sequence displayed.

"R" "Y" "M" "K" "W" and "S" designate nucleotide positions which are degenerated. "R" stands for A or G; "Y" stands for C or T; "M" stands for A or C; "K" stands for G or T; "W" stands for A or T; "S" stands for C or G. "I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.

[a] This sequence is the reverse-complement of the selected sequence.

Annex XXXI: Strategy for the selection of *Streptococcus pneumoniae*-specific amplification primers and hybridization probes from *pbp1a* sequences.

	Accession #	453 505 678 706	SEQ ID NO.:
pbp1a	M90528	A TTGACTAcCC AAGCATaCAc TATGCtAAtG CtATTTCAAG TAATACAACC GA...TATATG ATGACaGAtA TGATGAAAAC CGT...	-
pbp1a	X67873	A TCGACTAcCC AAGtATtCAc TActCAAAtG CCATTTCAAG TAAcACAACC GA...TATATG ATGACCGAAA TGATGAAAAC AGT...	-
pbp1a	AB006868	A TCGACTAcCC AAGtATtCAc TActCAAAtG CCATTTCAAG TAAcACAACC GA...TATATG ATGACCGACA TGATGAAAAC AGT...	-
pbp1a	AF046234	A TCGACTAcCC AAGtATtCAc TActCAAAtG CCATTTCAAG TAAcACAACC GA...TATATG ATGACCGAAA TGATGAAAAC TGT...	-
pbp1a		A TCGACTAcCC AAGtATtCAc TActCAAAtG CCATTTCAAG TAAcACAACC GA...TATATG ATGACCGACA TGATGAAAAC TGT...	1014
pbp1a		A TCGACTAcCC AAGtATtCAc TActCAAAtG CCATTTCAAG TAAcACAACC GA...TACATG ATGACCGAAA TGATGAAAAC TGT...	1017
pbp1a	AB006873	A TCGACTAcCC AAGtATtCAc TActCAAAtG CCATTTCAAG TAAcACAACC GA...TATATG ATGACCGACA TGATGAAAAC AGT...	-
pbp1a	AF139883	A TCGACTAcCC AAGtcTtCAc TActCAAAtG CCATTTCAAG TAAcACAACC GA...TATATG ATGACCGACA TGATGAAAAC AGT...	1169
pbp1a		A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAATACAACA GA...TATATG ATGACCGACA TGATGAAAAC AGT...	1004
pbp1a		A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAATACAACA GA...TATATG ATGACCGACA TGATGAAAAC AGT...	1007
pbp1a		A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAATACAACA GA...TATATG ATGACCGACA TGATGAAAAC AGT...	1008
pbp1a		A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAATACAACA GA...TATATG ATGACCGACA TGATGAAAAC AGT...	1009
pbp1a		A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAATACAACA GA...TATATG ATGACCGACA TGATGAAAAC AGT...	1011
pbp1a	AF159448	A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAATACAACA GA...TATATG ATGACCGACA TGATGAAAAC AGT...	-
pbp1a		A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAATACAACA GA...TACATG ATGACCGAAA TGATGAAAAC TGT...	1005
pbp1a		A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAATACAACA GA...TACATG ATGACCGAAA TGATGAAAAC TGT...	1015
pbp1a		A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAATACAACA GA...TACATG ATGACCGAAA TGATGAAAAC TGT...	1006
pbp1a		A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAATACAACA GA...TACATG ATGACCGAAA TGATGAAAAC TGT...	1012
pbp1a	X67867	A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAATACAACA GA...TACATG ATGACCGAAA TGATGAAAAC TGT...	-
pbp1a		A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAAcACAACT GA...TATATG ATGACtGAAA TGATGAAAAC TGT...	1010
pbp1a	Z49094	A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAAcACAACT GA...TATATG ATGACtGAAA TGATGAAAAC TGT...	-
pbp1a		A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAAcACAACT GA...TATATG ATGACtGAAA TGATGAAAAC TGT...	1013
pbp1a		A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAAcACAACT GA...TATATG ATGACtGAAA TGATGAAAAC TGT...	1016
pbp1a	X67870	A TCGACTATCC AAGtATGCAT TAcGCAAACG CCATTTCAAG TAAcACAACT GA...TATATG ATGACCGAAA TGATGAAAAC TGT...	-
pbp1a		A TTGACTATCC AAGtATtCAc TActCAAAtG CtATTTCAAG TAATACAACT GA...TATATG ATGACtGAAA TGATGAAAAC TGT...	1018
pbp1a	AJ002290	A TTGAtTAcCC AActATGgtc TATGCtAACG CtATTTCAAG TAATACAACT GA...TACATG ATGACtGAAA TGATGAAAAC AGT...	-
pbp1a	X67871	A TCGACTAcCC AAGtcTtCAc TActCAAAtG CCATTTCAAG TAAcACAACC GA...TACATG ATGACaGAAA TGATGAAAAC AGT...	-
Selected sequences for amplification primers		GACTATCC AAGCATGCAT TATG	1130
		ATG ATGACHGAMA TGATGAAAAC	1129
Selected sequence for hybridization probe		CAAACG CCATTTCAAG TAATACAAC	1197

The sequence numbering refers to the *Streptococcus pneumoniae pbp1a* gene fragment (SEQ ID NO. 1004). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower- case letters. Dotes indicate gaps in the sequences displayed.

"R" "Y" "M" "K" "W" and "S" designate nucleotide positions which are degenerated. "R" stands for A or G; "Y" stands for C or T; "M" stands for A or C; "K" stands for G or T; "W" stands for A or T; "H" stands for A, C or T;"S" stands for C or G. "I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.

Annex XXXI: Strategy for the selection of *Streptococcus pneumoniae*-specific amplification primers and hybridization probes from *pbp1a* sequ nc s (continued).

	Accession #	756 … 783 813 … 840	SEQ ID NO.:
pbp1a	M90528	...GCTGGTAA aACtGGTACg TCaAACTATA...A ATACgGGTTA TGTAGCTCCG GAcGAAA	-
pbp1a	X67873	...GCTGGTAA aACAGGaACc TCTAACTATA...A CCtCTcaaTt TGTAGCaCCt GATGAAC	-
pbp1a	AB006868	...GCTGGTAA aACAGGaACc TCTAACTATA...A CCtCTcaaTt TGTAGCaCCt GAcGAAC	-
pbp1a	AF046234	...GCAGGTAA aACAGGTACT TCTAACTATA...A ACACTGGTTA CGTAGCTCCA GATGAAA	-
pbp1a		...GCAGGTAA aACAGGTACT TCTAACTATA...A ACACTGGTTA CGTAGCTCCA GATGAAA	1014
pbp1a		...GCTGGTAA GACAGGTACT TCTAACTACA...A ACACTGGCTA TGTAGCTCCA GATGAAA	1017
pbp1a	AB006873	...GCAGGTAA GACAGGTACT TCTAACTATA...A ACACTGGCTA CGTAGCTCCA GATGAAA	-
pbp1a	AF139883	...GCTGGTAA aACAGGaACc TCTAACTATA...A ACACTGGCTA TGTAGCTCCA GATGAAA	1169
pbp1a		...GCTGGTAA aACAGGaACc TCTAACTATA...A ACACTGGCTA TGTAGCTCCA GATGAAA	1004
pbp1a		...GCTGGTAA aACAGGaACc TCTAACTATA...A ACACTGGCTA TGTAGCTCCA GATGAAA	1007
pbp1a		...GCTGGTAA aACAGGaACc TCTAACTATA...A ACACTGGCTA TGTAGCTCCA GATGAAA	1008
pbp1a		...GCTGGTAA aACAGGaACc TCTAACTATA...A ACACTGGCTA TGTAGCTCCA GATGAAA	1009
pbp1a		...GCTGGTAA aACAGGaACc TCTAACTATA...A ACACTGGCTA TGTAGCTCCA GATGAAA	1011
pbp1a	AF159448	...GCTGGTAA aACAGGaACc TCTAACTATA...A ACACTGGCTA TGTAGCTCCA GATGAAA	-
pbp1a		...GCTGGTAA GACAGGTACT TCTAACTACA...A ACACTGGCTA TGTAGCTCCA GATGAAA	1005
pbp1a		...GCTGGTAA GACAGGTACT TCTAACTACA...A ACACTGGCTA TGTAGCTCCA GATGAAA	1015
pbp1a		...GCTGGTAA GACAGGTACT TCTAACTACA...A ACACTGGCTA TGTAGCTCCA GATGAAA	1006
pbp1a		...GCTGGTAA GACAGGTACT TCTAACTACA...A ACACTGGCTA TGTAGCTCCA GATGAAA	1012
pbp1a	X67867	...GCTGGTAA GACAGGTACT TCTAACTACA...A ACACTGGCTA TGTAGCTCCA GATGAAA	-
pbp1a		...GCAGGTAA GACAGGTACT TCTAACTATA...A ACACTGGTTA CGTAGCTCCA GATGAAA	1010
pbp1a	Z49094	...GCAGGTAA GACAGGTACT TCTAACTATA...A ACACTGGTTA CGTAGCTCCA GATGAAA	-
pbp1a		...GCAGGTAA GACAGGTACT TCTAACTATA...A ACACTGGCTA CGTAGCTCCA GATGAAA	1013
pbp1a		...GCAGGTAA GACAGGTACT TCTAACTATA...A ACACTGGCTA CGTAGCTCCA GATGAAA	1016
pbp1a	X67870	...GCAGGTAA GACAGGTACT TCTAACTATA...A ACACTGGCTA CGTAGCTCCA GATGAAA	-
pbp1a		...GCAGGTAA GACAGGTACT TCTAACTATA...A ACACTGGCTA CGTAGCTCCA GATGAAA	1018
pbp1a	AJ002290	...GCAGGTAA GACgGGTACa TCTAACTACA...A ACACTGGCTA C--------- -------	-
pbp1a	X67871	...GCTGGTAA aACAGGTACc TCTAACTATA...A ACACTGGTTA CGTAGCTCCA GATGAAA	-
Selected sequence for hybridization probe		GGTAA GACAGGTACT TCTAACT	1193
Selected sequence for amplification primer°		ACTGGYTA YGTAGCTCCA GATG	1131

The sequence numbering refers to the *Streptococcus pneumoniae pbp1a* gene fragment (SEQ ID NO. 1004). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower- case letters. Dots indicate gaps in the sequences displayed. "-"indicates incomplete sequence data.

"R" "Y" "W" and "S" designate nucleotide positions which are degenerated. "R" stands for A or G; "Y" stands for C or T; "W" stands for A or T; "S" stands for C or G. "I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.

° This sequence is the reverse-complement of the selected primer.

Annex XXXII: Specific and ubiquitous primers for nucleic acid amplification (toxin sequences).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment	
		SEQ ID NO.	Nucleotide position
Toxin gene:	***cdtA***		
2123	5'-TCT ACC ACT GAA GCA TTA C	2129[a]	442-460
2124[b]	5'-TAG GTA CTG TAG GTT TAT TG	2129[a]	580-599
Toxin gene:	***cdtB***		
2126	5'-ATA TCA GAG ACT GAT GAG	2130[a]	2665-2682
2127[b]	5'-TAG CAT ATT CAG AGA ATA TTG T	2130[a]	2746-2767
Toxin gene:	***stx_1***		
1081	5'-ATG TCA GAG GGA TAG ATC CA	1076[a]	233-252
1080[b]	5'-TAT AGC TAC TGT CAC CAG ACA ATG T	1076[a]	394-418
Toxin gene:	***stx_2***		
1078	5'-AGT TCT GCG TTT TGT CAC TGT C	1077[a]	546-567
1079[b]	5'-CGG AAG CAC ATT GCT GAT T	1077[a]	687-705
Toxin genes:	***stx_1 and stx_2***		
1082	5'-TTG ARC RAA ATA ATT TAT ATG TG	1076[a]	278-300
1083[b]	5'-TGA TGA TGR CAA TTC AGT AT	1076[a]	781-800

[a] Sequences from databases.

[b] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex XXXIII: Molecular beacon internal hybridization probes for specific detection of toxin sequences.

SEQ ID NO.	Nucleotide sequence[a]	Originating DNA fragment	
		SEQ ID NO.	Nucleotide position
Toxin gene:	*cdtA*		
2125[b]	5'-CAC GCG GAT TTT GAA TCT CTT CCT CTA GTA GCG CGT G	2129[c]	462-488
Toxin gene:	*cdtB*		
2128	5'-CAA CGC TGG AGA ATC TAT ATT TGT AGA AAC TGC GTT G	2130[c]	2714-2740
Toxin gene:	*stx₁*		
1084	5'-CCA CGC CGC TTT GCT GAT TTT TCA CAT GTT ACC GCG TGG	1076[c]	337-363
2012[d]	5'-CCG CGG ATT ATT AAA CCG CCC TTC CGC GG-MR-HEG-ATG TCA GAG GGA TAG ATC CA	1076[c]	248-264
Toxin gene:	*stx₂*		
1085	5'-CCA CGC CAC TGT CTG AAA CTG CTC CTG TG CGT GG	1077[c]	617-638

[a] Underlined nucleotides indicate the molecular beacon's stem.

[b] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

[c] Sequences from databases.

[d] Scorpion primer.

Annex XXXIV: Specific and ubiquitous primers for nucl ic acid amplification (*van* sequenc s).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Nucleotide position
Resistance gene:	***vanA***		
1086	5'-CTA CTC CCG CCT TTT GGG TT	1049-1057[a]	513-532[b]
1087[c]	5'-CTC ACA GCC CGA AAC AGC CT	1049-1057[a]	699-718[b]
1086	5'-CTA CTC CCG CCT TTT GGG TT	1049-1057[a]	513-532[b]
1088[c]	5'-TGC CGT TTC CTG TAT CCG TC	1049-1057[a]	885-904[b]
1086	5'-CTA CTC CCG CCT TTT GGG TT	1049-1057[a]	513-532[b]
1089[c]	5'-ATC CAC ACG GGC TAG ACC TC	1049-1057[a]	933-952[b]
1090	5'-AAT AGC GCG GAC GAA TTG GAC	1049-1057[a]	629-649[b]
1091[c]	5'-AAC GCG GCA CTG TTT CCC AA	1049-1057[a]	734-753[b]
1090	5'-AAT AGC GCG GAC GAA TTG GAC	1049-1057[a]	629-649[b]
1089[c]	5'-ATC CAC ACG GGC TAG ACC TC	1049-1057[a]	933-952[b]
1092	5'-TCG GCA AGA CAA TAT GAC AGC	1049-1057[a]	662-682[b]
1088[c]	5'-TGC CGT TTC CTG TAT CCG TC	1049-1057[a]	885-904[b]
Resistance gene:	***vanB***		
1095	5'-CGA TAG AAG CAG CAG GAC AA	1117[d]	473-492
1096[c]	5'-CTG ATG GAT GCG GAA GAT AC	1117[d]	611-630
Resistance genes:	***vanA, vanB***		
1112	5'-GGC TGY GAT ATT CAA AGC TC	1049-1057,1117[a]	437-456[b]
1113[c]	5'-ACC GAC CTC ACA GCC CGA AA	1049-1057,1117[a]	705-724[b]
1112	5'-GGC TGY GAT ATT CAA AGC TC	1049-1057,1117[a]	437-456[b]
1114[c]	5'-TCW GAG CCT TTT TCC GGC TCG	1049-1057,1117[a]	817-837[b]
1115	5'-TTT CGG GCT GTG AGG TCG GBT GHG CG	1049-1057,1117[a]	705-730[b]
1114[c]	5'-TCW GAG CCT TTT TCC GGC TCG	1049-1057,1117[a]	817-837[b]
1116	5'-TTT CGG GCT GTG AGG TCG GBT GHG CGG	1049-1057,1117[a]	705-731[b]
1114[c]	5'-TCW GAG CCT TTT TCC GGC TCG	1049-1057,1117[a]	817-837[b]
1112	5'-GGC TGY GAT ATT CAA AGC TC	1049-1057,1117[a]	437-456[b]
1118[c]	5'-TTT TCW GAG CCT TTT TCC GGC TCG	1049-1057,1117[a]	817-840[b]

[a] These sequences were aligned to derive the corresponding primer.
[b] The nucleotide positions refer to the *vanA* sequence fragment (SEQ ID NO. 1051).
[c] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.
[d] Sequences from databases.

Annex XXXIV: Specific and ubiquitous primers for nucleic acid amplification (van s quences) (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment: SEQ ID NO.	Originating DNA fragment: Nucleotide position
Resistance genes:	***vanA, vanB* (continued)**		
1115	5'-TTT CGG GCT GTG AGG TCG GBT GHG CG	1049-1057,1117[a]	705-730[b]
1118[c]	5'-TTT TCW GAG CCT TTT TCC GGC TCG	1049-1057,1117[a]	817-840[b]
1116	5'-TTT CGG GCT GTG AGG TCG GBT GHG CGG	1049-1057,1117[a]	705-731[b]
1118[c]	5'-TTT TCW GAG CCT TTT TCC GGC TCG	1049-1057,1117[a]	817-840[b]
1119	5'-TTT CGG GCT GTG AGG TCG GBT GHG C	1049-1057,1117[a]	705-729[b]
1118[c]	5'-TTT TCW GAG CCT TTT TCC GGC TCG	1049-1057,1117[a]	817-840[b]
1120	5'-TTT CGG GCT GTG AGG TCG GBT GHG	1049-1057,1117[a]	705-728[b]
1118[c]	5'-TTT TCW GAG CCT TTT TCC GGC TCG	1049-1057,1117[a]	817-840[b]
1121	5'-TGT TTG WAT TGT CYG GYA TCC C	1049-1057,1117[a]	408-429[b]
1111[c]	5'-CTT TTT CCG GCT CGW YTT CCT GAT G	1049-1057,1117[a]	806-830[b]
1112	5'-GGC TGY GAT ATT CAA AGC TC	1049-1057,1117[a]	437-456[b]
1111[c]	5'-CTT TTT CCG GCT CGW YTT CCT GAT G	1049-1057,1117[a]	806-830[b]
1123	5'-TTT CGG GCT GTG AGG TCG GBT G	1049-1057,1117[a]	705-726[b]
1111[c]	5'-CTT TTT CCG GCT CGW YTT CCT GAT G	1049-1057,1117[a]	806-830[b]
1112	5'-GGC TGY GAT ATT CAA AGC TC	1049-1057,1117[a]	437-456[b]
1124[c]	5'-GAT TTG RTC CAC YTC GCC RAC A	1049-1057,1117[a]	757-778[b]
Resistance gene:	***vanC1***		
1103	5'-ATC CCG CTA TGA AAA CGA TC	1058-1059[a]	519-538[d]
1104[c]	5'-GGA TCA ACA CAG TAG AAC CG	1058-1059[a]	678-697[d]
Resistance genes:	***vanC1, vanC2, vanC3***		
1097	5'-TCY TCA AAA GGG ATC ACW AAA GTM AC	1058-1066[a]	607-632[d]
1098[c]	5'-TCT TCA AAA TCG AAA AAG CCG TC	1058-1066[a]	787-809[d]
1099	5'-TCA AAA GGG ATC ACW AAA GTM AC	1058-1066[a]	610-632[d]
1100[c]	5'-GTA AAK CCC GGC ATR GTR TTG ATT TC	1058-1066[a]	976-1001[d]
1101	5'-GAC GGY TTT TTY GAT TTT GAA GA	1058-1066[a]	787-809[d]
1102[c]	5'-AAA AAR TCG ATK CGA GCM AGA CC	1058-1066[a]	922-944[d]
Resistance genes:	***vanC2, vanC3***		
1105	5'-CTC CTA CGA TTC TCT TGA YAA ATC A	1060-1066,1140[a]	487-511[e]
1106[c]	5'-CAA CCG ATC TCA ACA CCG GCA AT	1060-1066,1140[a]	690-712[e]

[a] These sequences were aligned to derive the corresponding primer.
[b] The nucleotide positions refer to the *vanA* sequence fragment (SEQ ID NO. 1051).
[c] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.
[d] The nucleotide positions refer to the *vanC1* sequence fragment (SEQ ID NO. 1058).
[e] The nucleotide positions refer to the *vanC2* sequence fragment (SEQ ID NO. 1140).

Annex XXXIV: Specific and ubiquitous primers for nucleic acid amplification (*van* sequences) (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Resistance gene:	***vanD***		
1591	5'-ATG AGG TAA TAG AAC GGA TT	1594	797-837
1592[b]	5'-CAG TAT TTC AGT AAG CGT AAA	1594	979-999
Resistance gene:	***vanE***		
1595	5'-AAA TAA TGC TCC ATC AAT TTG CTG A	1599[a]	74-98
1596[b]	5'-ATA GTC GAA AAA GCC ATC CAC AAG	1599[a]	394-417
1597	5'-GAT GAA TTT GCG AAA ATA CAT GGA	1599[a]	163-186
1598[b]	5'-CAG CCA ATT TCT ACC CCT TTC AC	1599[a]	319-341
	Sequencing primers (*vanAB*)		
1112	5'-GGC TGY GAT ATT CAA AGC TC	1139[a]	737-756
1111[b]	5'-CTT TTT CCG GCT CGW YTT CCT GAT G	1139[a]	1106-1130
	Sequencing primers (*vanA, vanX, vanY*)		
1150	5'-TGA TAA TCA CAC CGC ATA CG	1141[a]	860-879
1151[b]	5'-TGC TGT CAT ATT GTC TTG CC	1141[a]	1549-1568
1152	5'-ATA AAG ATG ATA GGC CGG TG	1141[a]	1422-1441
1153[b]	5'-CTC GTA TGT CCC TAC AAT GC	1141[a]	2114-2133
1154	5'-GTT TGA AGC ATA TAG CCT CG	1141[a]	2520-2539
1155[b]	5'-CAG TGC TTC ATT AAC GTA GTC	1141[a]	3089-3109

[a] Sequences from databases.
[b] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex XXXIV: Sp cific and ubiquitous primers for nucleic acid amplification (*van* sequences) (continued).

SEQ ID NO.	Nucleotide sequence SEQ ID	Originating DNA fragment	
		Nucleotide NO.	position
	Sequencing primers (*vanC1*)		
1110	5'-ACG AGA AAG ACA ACA GGA AGA CC	1138[a]	122-144
1109[b]	5'-ACA TCG TGA TCG CTA AAA GGA GC	1138[a]	1315-1337
	Sequencing primers (*vanC2*, *vanC3*)		
1108	5'-GTA AGA ATC GGA AAA GCG GAA GG	1140[a]	1-23
1107[b]	5'-CTC ATT TGA CTT CCT CCT TTG CT	1140[a]	1064-1086

[a] Sequences from databases.

[b] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex XXXV: Internal hybridization probes for sp cific detection of van sequences.

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Nucleotide position
Resistance gene:	***vanA***		
1170	5'-ACG AAT TGG ACT ACG CAA TT	1049-1057[a]	639-658[b]
2292	5'-GAA TCG GCA AGA CAA TAT G	2293[c]	583-601
Resistance gene:	***vanB***		
1171	5'-ACG AGG ATG ATT TGA TTG TC	1117[c]	560-579
2294	5'-AAA CGA GGA TGA TTT GAT TG	2296[a]	660-679
2295	5'-TTG AGC AAG CGA TTT CGG	2296[a]	614-631
Resistance gene:	***vanD***		
2297	5'-TTC AGG AGG GGG ATC GC	1594[c]	458-474

[a] These sequences were aligned to derive the corresponding primer.
[b] The nucleotide positions refer to the *vanA* sequence fragment (SEQ ID NO. 1051).
[c] Sequences from databases.

Annex XXXVI: Specific and ubiquitous primers for nucleic acid amplification (*pbp* sequences).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID. NO.	Originating DNA fragment Nucleotide position
<u>Resistance gene</u>:	***pbp1a***		
1129	5'-ATG ATG ACH GAM ATG ATG AAA AC	1004-1018[a]	681-703[b]
1131[c]	5'-CAT CTG GAG CTA CRT ARC CAG T	1004-1018[a]	816-837[b]
1130	5'-GAC TAT CCA AGC ATG CAT TAT G	1004-1018[a]	456-477[b]
1131	5'-CAT CTG GAG CTA CRT ARC CAG T	1004-1018[a]	816-837[b]
2015	5'-CCA AGA AGC TCA AAA ACA TCT G	2047[d]	909-930
2016[c]	5'-TAD CCT GTC CAW ACA GCC AT	2047[d]	1777-1796
	Sequencing primers (*pbp1a*)		
1125	5'-ACT CAC AAC TGG GAT GGA TG	1169[d]	873-892
1126[c]	5'-TTA TGG TTG TGC TGG TTG AGG	1169[d]	2140-2160
1125	5'-ACT CAC AAC TGG GAT GGA TG	1169[d]	873-892
1128[c]	5'-GAC GAC YTT ATK GAT ATA CA	1169[d]	1499-1518
1127	5'-KCA AAY GCC ATT TCA AGT AA	1169[d]	1384-1403
1126[c]	5'-TTA TGG TTG TGC TGG TTG AGG	1169[d]	2140-2160
	Sequencing primers (*pbp2b*)		
1142	5'-GAT CCT CTA AAT GAT TCT CAG GTG G	1172[d]	1-25
1143[c]	5'-CAA TTA GCT TAG CAA TAG GTG TTG G	1172[d]	1481-1505
1142	5'-GAT CCT CTA AAT GAT TCT CAG GTG G	1172[d]	1-25
1145[c]	5'-AAC ATA TTK GGT TGA TAG GT	1172[d]	793-812
1144	5'-TGT YTT CCA AGG TTC AGC TC	1172[d]	657-676
1143[c]	5'-CAA TTA GCT TAG CAA TAG GTG TTG G	1172[d]	1481-1505
	Sequencing primers (*pbp2x*)		
1146	5'-GGG ATT ACC TAT GCC AAT ATG AT	1173[d]	219-241
1147[c]	5'-AGC TGT GTT AGC VCG AAC ATC TTG	1173[d]	1938-1961
1146	5'-GGG ATT ACC TAT GCC AAT ATG AT	1173[d]	219-241
1149[c]	5'-TCC YAC WAT TTC TTT TTG WG	1173[d]	1231-1250
1148	5'-GAC TTT GTT TGG CGT GAT AT	1173[d]	711-730
1147[c]	5'-AGC TGT GTT AGC VCG AAC ATC TTG	1173[d]	1938-1961

[a] These sequences were aligned to derive the corresponding primer.

[b] The nucleotide positions refer to the *pbp1a* sequence fragment (SEQ ID NO. 1004).

[c] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

[d] Sequences from databases.

Annex XXXVII: Internal hybridization probes for specific detection of *pbp* s quences.

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment	
		SEQ ID NO.	Nucleotide position
Resistance gene:	***pbp*1a**		
1132	5'-AGT GAA AAR ATG GCT GCT GC	1004-1018[a]	531-550[b]
1133	5'-CAT CAA GAA CAC TGG CTA YGT AG	1004-1018[a]	806-828[b]
1134	5'-CTA GAT AGA GCT AAA ACC TTC CT	1004-1018[a]	417-439[b]
1135	5'-CAT TAT GCA AAC GCC ATT TCA AG	1004-1018[a]	471-493[b]
1192	5'-GGT AAA ACA GGA ACC TCT AAC T	1004-1018[a]	759-780[b]
1193	5'-GGT AAG ACA GGT ACT TCT AAC T	1004-1018[a]	759-780[b]
1194	5'-CAT TTC AAG TAA TAC AAC AGA ATC	1004-1018[a]	485-508[b]
1195	5'-CAT TTC AAG TAA CAC AAC TGA ATC	1004-1018[a]	485-508[b]
1196	5'-GCC ATT TCA AGT AAT ACA ACA GAA	1004-1018[a]	483-506[b]
1197	5'-CAA ACG CCA TTT CAA GTA ATA CAA C	1004-1018[a]	478-502[b]
1094	5'-GGT AAA ACA GGT ACT TCT AAC TA	1004-1018[a]	759-781[b]
1214	5'-GGT AAA ACA GGT ACC TCT AAC TA	1004-1018[a]	759-781[b]
1216	5'-GGT AAG ACT GGT ACA TCA AAC TA	1004-1018[a]	759-781[b]
1217	5'-CAA ATG CCA TTT CAA GTA ACA CAA C	1004-1018[a]	478-502[b]
1218	5'-CAA ACG CCA TTT CAA GTA ACA CAA C	1004-1018[a]	478-502[b]
1219	5'-CAA ATG CTA TTT CAA GTA ATA CAA C	1004-1018[a]	478-502[b]
1220	5'-CAA ACG CCA TTT CAA GTA ATA CGA C	1004-1018[a]	478-502[b]
2017	5'-ACT TTG AAT AAG GTC GGT CTA G	2047[c]	1306-1327
2018	5'-ACA CTA AAC AAG GTT GGT TTA G	2063	354-375
2019	5'-ACA CTA AAC AAG GTC GGT CTA G	2064	346-367
2020	5'-GTA GCT CCA GAT GAA ATG TTT G	2140[c]	1732-1753
2021	5'-GTA GCT CCA GAC GAA ATG TTT G	2057	831-852
2022	5'-GTA GCT CCA GAT GAA ACG TTT G	2053[c]	805-826
2023	5'-GTA ACT CCA GAT GAA ATG TTT G	2056	819-840
2024	5'-AGT GAA AAG ATG GCT GCT GC	2048[c]	1438-1457
2025	5'-AGT GAG AAA ATG GCT GCT GC	2047[c]	1438-1457
2026	5'-TCC AAG CAT GCA TTA TGC AAA CG	2047[c]	1368-1390
2027	5'-TCG GTC TAG ATA GAG CTA AAA CG	2047[c]	1319-1341
2028	5'-TAT GCT CTT CAA CAA TCA CG	2047[c]	1267-1286
2029	5'-AGC CGT TGA GAC TTT GAA TAA G	2047[c]	1296-1317
2030	5'-CTT AAT GGT CTT GGT ATC G	2047[c]	1345-1366
2031	5'-CGT GAC TGG GGT TCT GCT ATG A	2049[c]	1096-1117
2032	5'-CGT GAC TGG GGA TCA TCA ATG A	2047[c]	1096-1117
2033	5'-CGT GAC TGG GGT TCT GCC ATG A	2057	195-216
2034	5'-ATC AAG AAC ACT GGC TAT GTA G	2050[c]	787-808

[a] These sequences were aligned to derive the corresponding primer.
[b] The nucleotide positions refer to the *pbp*1a sequence fragment (SEQ ID NO. 1004).
[c] Sequence from databases.

Annex XXXVII: Internal hybridization probes for specific detection of *pbp* sequences (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment	
		SEQ ID NO.	Nucleotide position
<u>Resistance gene</u>:	***pbp1a* (continued)**		
2035	5'-ATC AAG AAC ACT GGC TAC GTA G	2051[c]	787-808
2036	5'-ATC AAG AAC ACT GGT TAC GTA G	2047	1714-1735
2037	5'-ATC AAA AAT ACT GGT TAT GTA G	2057	813-834
2038	5'-ATC AAG AAT ACT GGC TAC GTA G	2052[c]	757-778
2039	5'-ATC AAA AAC ACT GGC TAT GTA G	2053[c]	787-808

Ann x XXXVIII: Strategy fo the selection of *vanAB*-specific amplification prim rs and *vanA*- and *va* nB- specific hybridization probes from *van* sequences.

	Accession #	734	759 936 961	SEQ ID NO.:
vanA	X56895	GTAGGCT GCGATAT		1139
vanA	M97297	GTAGGCT GCGATAT	CA AAGCTCAGC...CGGACGAATT GGACTACGCA ATTGAA...	1141
vanA		GTAGGCT GCGATAT	CA AAGCTCAGC...CGGACGAATT GGACTACGCA ATTGAA...	1051
vanA		GTAGGCT GCGATAT	CA AAGCTCAGC...CGGACGAATT GGACTACGCA ATTGAA...	1052
vanA		GTAGGCT GCGATAT	CA AAGCTCAGC...CGGACGAATT GGACTACGCA ATTGAA...	1053
vanA		GTAGGCT GCGATAT	CA AAGCTCAGC...CGGACGAATT GGACTACGCA ATTGAA...	1054
vanA		GTAGGCT GCGATAT	CA AAGCTCAGC...CGGACGAATT GGACTACGCA ATTGAA...	1055
vanA		GTAGGCT GCGATAT	CA AAGCTCAGC...CGGACGAATT GGACTACGCA ATTGAA...	1056
vanA		GTAGGCT GCGATAT	CA AAGCTCAGC...CGGACGAATT GGACTACGCA ATTGAA...	1057
vanA		GTAGGCT GCGATAT	CA AAGCTCAGC...CGGACGAATT GGACTACGCA ATTGAA...	1049
vanA		GTAGGCT GCGATAT	CA AAGCTCAGC...CGGACGAATT GGACTACGCA ATTGAA...	1050
vanB	U94526	GTGGGCT GTGATAT	CA AAGCTCAGC...CGGACGAATT GGACTACGCA ATTGAA...	1117
vanB	U94527	GTAGGCT GCGATAT	CA AAGCTCCGC...CGGAaGAAcT taACgctGCg ATaGAA...	-
vanB	U94528	GTGGGCT GTGATAT	CA AAGCTCCGC...CGGAaGAAcT aaACgctGCg ATaGAA...	-
vanB	U94529	GTGGGCT GTGATAT	CA AAGCTCCGC...CGGAaGAAcT taACgctGCg ATaGAA...	-
vanB	U94530	GTGGGCT GTGATAT	CA AAGCTCCGC...CGGAaGAAcT taACgctGCg ATaGAA...	-
vanB	Z83305	GTGGGCT GTGATAT	CA AAGCTCCGC...CGGAaGAAcT taACgctGCg ATaGAA...	-
vanB	U81452	GTGGGCT GTGATAT	CA AAGCTCCGC...CGGAaGAAcT taACgctGCg ATaGAA...	-
vanB	U35369	GTAGGCT GCGATAT	CA AAGCTCCGC...CGGAaGAAcT taACgctGCg ATaGAA...	-
vanB	U72704	GTGGGCT GCGATAT	CA AAGCTCCGC...CGGAaGAAcT aaACgctGCg ATaGAA...	-
vanB	L06138	GTAGGCT GCGATAT	CA AAGCTCCGC...CGGAaGAAcT taACgctGCg ATaGAA...	-
vanB	L15304	GTGGGCT GTGATAT	CA AAGCTCCGC...CGGAaGAAcT aaACgctGCg ATaGAA...	-
vanB	U00456	GTAGGCT GCGATAT	CA AAGCTCCGC...CGGAaGAAcT taACgctGCg ATaGAA...	-
vanD	AF130997	GTGGGaT GCGATAT	CA AAGCTCCGC...CGGAaGAAcT aaACgctGCg ATaGAA...	-
vanE	AF136925	GTAGGtT GTGgTAT	CA AAGCTCCGT...CAGAaGAAcT GcAggcaGCA ATcGAA...	-
			gg AgctgCAGC...AAAgtGAtTT atAtaAAGCA ATaGAC...	
Selected sequence for amplification primer		GGCT GYGATAT	CA AAGCTC	1112
Selected sequence for hybridization probe			ACGAATT GGACTACGCA ATT (*vanA*)	1170

The sequence numbering refers to the *Ente rococcus faecium vanA* gene fragment (SEQ ID NO. 1139). Nucleotides in capitals are identical to the selected sequences or match those s equences. Mismatches are indicated by lower-case letters. Dots indicate gaps in the sequences displayed.

"R" "Y" "M" "K" "W" and "S" designate nu cleotide positions which are degenerated. "R" stands for A or G; "Y" stands for C or T; "M" stands for A or C; "K" stands for G or T; "W" stands for A or T; "S" stands for C or G. "I" stands for inosine which is a nucleotide analog that can bind to any of the four nu cleotides A, C, G or T.

Annex XXXVIII: Strategy for the selection of *vanAB*-specific amplification primers and *vanA*- and *vanB*- specific hybridization probes from *van* sequences (continued).

	Accession #	1038 ... 1063 1103 ... 1133	SEQ ID NO.:
vanA	X56895	GAAACagt GccGcGTTag TTGTtGGC...ATT CATCAGGAAG TCGAGCCGGA AAAAGGCT	1139
vanA	M97297	GAAACagt GccGcgTTag TTGTtGGC...ATT CATCAGGAAG TCGAGCCGGA AAAAGGCT	1141
vanA		GAAACagt GccGcgTTag TTGTtGGC...ATT CATCAGGAAG TCGAGCCGGA AAAAGGCT	1051
vanA		GAAACagt GccGcgTTag cTGTtGGC...ATT CATCAGGAAG TCGAGCCGGA AAAAGGCT	1052
vanA		GAAACagt GccGcgTTag cTGTtGGC...ATT CATCAGGAAG TCGAGCCGGA AAAAGGCT	1053
vanA		GAAACagt GccGcgTTag TTGTtGGC...ATT CATCAGGAAG TCGAGCCGGA AAAAGGCT	1054
vanA		GAAACagt GccGcgTTag cTGTtGGC...ATT CATCAGGAAG TCGAGCCGGA AAAAGGCT	1055
vanA		GAAACagt GccGcgTTag cTGTtGGC...ATT CATCAGGAAG TCGAGCCGGA AAAAGGCT	1056
vanA		GAAACagt GccGcgTTag cTGTtGGC...ATT CATCAGGAAG TCGAGCCGGA AAAAGGCT	1057
vanA		GAAACagt GccGcgTTag cTGTtGGC...ATT CATCAGGAAG TCGAGCCGGA AAAAGGCT	1049
vanA		GAAACagt GccGcgTTag cTGTtGGC...ATT CATCAGGAAG TCGAGCCGGA AAAAGGCT	1050
vanB	U94526	CGAACGAG GATGATTTGA TTGTCGGC...ATC CATCAGGAAA ACGAGCCGGA AAAAGGCT	1117
vanB	U94527	GAAACGAG GATGATTTGA TTGTCGGC...ATC CATCAGGAAA ACGAGCCGGA AAAAGGCT	-
vanB	U94528	GGAACGAG GATGATTTGA TTGTCGGC...ATC CATCAGGAAA ACGAGCCGGA AAAAGGCT	-
vanB	U94529	GGAACGAG GATGATTTGA TTGTCGGC...ATC CATCAGGAAA ACGAGCCGGA AAAAGGCT	-
vanB	U94530	GGAACGAG GATGATTTGA TTGTCGGC...ATC CATCAGGAAA ACGAGCCGGA AAAAGGCT	-
vanB	Z83305	GGAACGAG GATGATTTGA TTGTCGGC...ATC CATCAGGAAA ACGAGCCGGA AAAAGGCT	-
vanB	U81452	GAAACGAG GATGATTTGA TTGTCGGC...ATC CATCAGGAAA ACGAGCCGGA AAAAGGCT	-
vanB	U35369	GGAACGAG GATGATTTGA TTGTCGGC...ATC CATCAGGAAA ACGAGCCGGA AAAAGGAT	-
vanB	U72704	GAAACGAG GATGATTTGA TTGTCGGC...ATC CATCAGGAAA ACGAGCCGGA AAAAGGCT	-
vanB	L06138	GGAACGAG GATGATTTGA TTGTCGGC...ATC CATCAGGAAA ACGAGCCGGA AAAAGGCT	-
vanB	L15304	GAAACGAG GATGATTTGA TTGTCGGC...ATC CATCAGGAAA ACGAGCCGGA AAAAGGCT	-
vanB	U00456	GAAACGga aATGATcTcA TgGctGGC...ATT CATCAGGAAG cacAGCCGGA aAAGGGAT	-
vanD	AF130997	GGAA...t GAacAaTTGg TcGTtGGA...TAT gAagAGaAAt ACaA...... ......TT	-
vanE	AF136925		-

Selected sequence for hybridization probe: ACGAG GATGATTTGA TTGTC (*vanB*) — 1171

Selected sequence for amplification primer[a]: CATCAGGAAR WCGAGCCGGA AAAAG — 1111

The sequence numbering refers to the *Enterococcus faecium vanA* gene fragment (SEQ ID NO. 1139). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. Dots indicate gaps in the sequences displayed.

"R" and "W" designate nucleotide positions which are degenerated. "R" stands for A or G; "W" stands for A or T

[a] This sequence is the reverse-complement of the above selected primer.

Annex XXXIX: Int rnal hybridization probe for specific detection of *mecA*.

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment	
		SEQ ID NO.	Nucleotide position
Resistance gene:	***mecA***		
1177	5'-GCT CAA CAA GTT CCA GAT TA	1178[a]	1313-1332

[a] Sequence from databases.

Annex XL: Specific and ubiquitous primers for nucleic acid amplification (*hexA* sequences).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Bacterial species:	***Streptococcus pneumoniae***		
1179	5'-ATT TGG TGA CGG GTG ACT TT	1183[a]	431-450
1181[b]	5'-AGC AGC TTA CTA GAT GCC GT	1183-1191[c]	652-671[d]
	Sequencing primers		
1179	5'-ATT TGG TGA CGG GTG ACT TT	1183[a]	431-450
1182[b]	5'-AAC TGC AAG AGA TCC TTT GG	1183[a]	1045-1064

[a] Sequences from databases.

[b] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

[c] These sequences were aligned to derive the corresponding primer.

[d] The nucleotide positions refer to the *hexA* sequence fragment (SEQ ID NO. 1183).

Annex XLI: Internal hybridization probe for specific detection of *hexA* sequences.

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment	
		SEQ ID NO.	Nucleotide position
Bacterial species:	***Streptococcus pneumoniae***		
1180[a]	5'-TCC ACC GTT GCC AAT CGC A	1183-1191[b]	629-647[c]

[a] This sequences is from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

[b] These sequences were aligned to derive the corresponding primer.

[c] The nucleotide positions refer to the *hexA* sequence fragment (SEQ ID NO. 1183).

Annex XLII: Strategy for the selection of *Streptococcus pneumoniae* species-specific amplification primers and hybridization probe from *hexA* sequences.

	428	453 626 674 1042 1067	SEQ ID NO.:
S. pneumoniae	TGG ATTTGGTGAC GGGTGACTTT	TAT...ATTTG CGATTGGCAA CGGTGGAGCA AACGGCATCT AGTAAGCTGC TCCA...AATCCAAAG GATCTCTTGC AGTTGGC	1183
S. pneumoniae	--- ------TGAC GGGTGACTTT	TAT...ATTTG CGATTGGCAA CGGTGGAGCA AACGGCATCT AGTAAGCTGC TCCA...AATCCAAAG GATCTCTTG- -------	1184
S. pneumoniae	--- ------TGAC GGGTGACTTT	TAT...ATTTG CGATTGGCAA CGGTGGAGCA AACGGCATCT AGTAAGCTGC TCCA...AATCCAAAG GATCTCT--- -------	1185
S. pneumoniae	--- ------TGAC GGGTGACTTT	TAT...ATTTG CGATTGGCAA CGGTGGAGCA AACGGCATCT AGTAAGCTGC TCCA...AATCCAAAG GATCTCTT-- -------	1186
S. pneumoniae	--- ------TGAC GGGTGACTTT	TAT...ATTTG CGATTGGCAA CGGTGGAGCA AACGGCATCT AGTAAGCTGC TCCG...AATCCAAAG GATCTCTT-- -------	1187
S. oralis	--- ---------- GGGTGACTTT	TAT...ATCca CGAcTGGCAg CtGTGGAGCA AgCGGCAgCT AGTAAGCTcC TCCA...---------- ---------- -------	1188
S. mitis	--- ----GGTGAC GGGTGACTTT	TAT...ATTca CGATTGGCAg CtGTGGAGCA AgCGGCATCT AGTAAaCTGC TTCA...AATCCAAAG GATCTCTT-- -------	1189
S. mitis	--- ------TGAC GGGTGACTTT	CAG...GCGaG gagcTGtCtc CtaTGGAGCG TcaGGCAgCa gGgAAaCTGC TGGA...---------- ---------- -------	1190
S. mitis	--- ------TGAC GGGTGACTTT	CAG...GCGaG gaAcTGtCtc CtaTGGAGCG TcaGGCAgCg gGgAAatTGC TAGA...AATCCAAAG GATCTCTT-- -------	1191
Selected sequence for amplification primer	ATTTGGTGAC GGGTGACTTT		1179
Selected sequences for amplification primers[a]		ACGGCATCT AGTAAGCTGC T	1181
		CCAAAG GATCTCTTGC AGTT	1182
Selected sequence for hybridization probe[a]		TG CGATTGGCAA CGGTGGA	1180

The sequence numbering refers to the *Streptococcus pneumoniae hexA* gene fragment (SEQ ID NO. 1183). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. Dots indicate gaps in the sequences displayed. "-" indicate incomplete sequence data.

[a] This sequence is the reverse-complement of the selected primer.

Annex XLIII: Specific and ubiquitous primers for nucleic acid amplification (*pcp* sequence).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Bacterial species:	***Streptococcus pyogenes***		
1211	5'-ATT CTT GTA ACA GGC TTT GAT CCC	1215[a]	291-314
1210[b]	5'-ACC AGC TTG CCC AAT ACA AAG G	1215[a]	473-494

[a] Sequences from databases.

[b] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex XLIV: Sp cific and ubiquitous primers for nucleic acid amplification of *S. saprophyticus* sequences of unknown coding potential.

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment	
		SEQ ID NO.	Nucleotide position
Bacterial species:	***Staphylococcus saprophyticus***		
1208	5'-TCA AAA AGT TTT CTA AAA AAT TTA C	74,1093, 1198[b]	169-193[c]
1209[a]	5'-ACG GGC GTC CAC AAA ATC AAT AGG A	74,1093, 1198[b]	355-379[c]

a This sequence is from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

b These sequences were aligned to derive the corresponding primer.

c The nucleotide positions refer to the *S. saprophyticus* unknown gene sequence fragment (SEQ ID NO. 1198).

Annex XLV: Molecular beacon internal hybridization probes for specific d tection of antimicrobial ag nts resistance gene sequences.

SEQ ID NO.	Nucleotide sequence[a]	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Resistance gene:	***gyrA***		
2250	5'-CCG TCG GAT GGT GTC GTA TAC CGC GGA GTC GCC GAC GG	1954[b]	218-243
2251	5'-CGG AGC CGT TCT CGC TGC GTT ACA TGC TGG TGG CTC CG	1954[b]	259-286
Resistance gene:	***mecA***		
1231	5'-GCG AGC CCG AAG ATA AAA AAG AAC CTC TGC TGC TCG C	1178[b]	1291-1315
Resistance gene:	***parC***		
1938[b]	5'-CCG CGC ACC ATT GCT TCG TAC ACT GAG GAG TCT CCG CGC GG	1321[c]	232-260
1939	5'-CGA CCC GGA TGG TAG TAT CGA TAA TGA TCC GCC AGC GGC CGG GTC G	1321[c]	317-346
1955[b]	5'-CGC GCA ACC ATT GCT TCG TAC ACT GAG GAG TCT GCG CG	1321[c]	235-260
Resistance gene:	***vanA***		
1239	5'-GCG AGC GCA GAC CTT TCA GCA GAG GAG GCT CGC	1051	860-880
1240	5'-GCG AGC CGG CAA GAC AAT ATG ACA GCA AAA TCG CTC GC	1051	663-688
Resistance gene:	***vanB***		
1241	5'-GCG AGC GGG GAA CGA GGA TGA TTT GAT TGG CTC GC	1117	555-577
Resistance gene:	***vanD***		
1593	5'-CCG AGC GAT TTA CCG GAT ACT TGG CTG ICG CTC GG	1594	835-845

a Underlined nucleotides indicate the molecular beacon's stem.
b This sequence is from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.
c Sequence from databases.

Annex XLVI: Molecular beacon internal hybridization probe for specific detection of *S. aureus* gene sequences of unknown coding pot ntial.

SEQ ID NO.	Nucleotide sequence[a]	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Bacterial species: ***S. aureus***			
1232	5'-GGA GCC GCG CGA TTT TAT AAA TGA ATG TTG ATA ACC GGC TCC	1244	53-80

[a] Underlined nucleotides indicate the molecular beacon's stem.

Annex XLVII: Molecular beacon internal hybridization probes for specific det ction of *tuf* sequences.

SEQ ID NO.	Nucleotide sequence[a]	Originating DNA fragment: SEQ ID NO.	Originating DNA fragment: Nucleotide position
Bacterial species:	***Chlamydia pneumoniae***		
2091	5'-CGC GAC TTG AGA TGG AAC TTA GTG AGC TTC TTG GTC GCG	20	157-183
2092	5'-CGC GAC GAA AGA ACT TCC TGA AGG TCG TGC AGG TCC AG	20	491-516
Bacterial species:	***Chlamydia trachomatis***		
2213	5'-CGT GCC ATT GAC ATG ATT TCC GAA GAA GAC GCT GAA GGC ACG	1739[b]	412-441
Bacterial species:	***Enterococcus faecalis***		
1236	5'-GCG AGC CGT GGT GAA GTT CGC GTT GGT GGC TCG C	883	370-391
Bacterial species:	***Enterococcus faecium***		
1235	5'-GCG AGC CGA AGT TGA AGT TGT TGG TAT TGC TGG CTC GC	64	412-437
Bacterial species:	***Legionella pneumophila***		
2084[c]	5'-CAC GCG TCA ACA CCC GTA CAA GTC GTC TTT TGC GCG TG	112	461-486
Bacterial species:	***Mycoplasma pneumoniae***		
2096[c]	5'-CGC GAC CGG TAC CAC GGC CAG TAA TCG TGT CGC G	2097[b]	658-679
Bacterial species:	***Neisseria gonorrhoeae***		
2177	5'-GGC ACG GAC AAA CCA TTC CTG CTG CCT ATC GAA ACG TGT TCC CGT GCC	126	323-357
2178	5'-GGC ACG ACA AAC CAT TCC TGC TGC CTA TCG AAC GTG CC	126	323-348
2179	5'-GGC AGC TCT ACT TCC GTA CCA CTG ACG TAA CCG GCT GCC	126	692-718

[a] Underlined nucleotides indicate the molecular beacon's stem.

[b] Sequence from databases.

[c] This sequence is from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex XLVII: Molecular b acon internal hybridization probes for specific d t ction of tuf sequences (continued).

SEQ ID NO.	Nucleotide sequence[a]	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Bacterial species:	***Pseudomonas aeruginosa***		
2122	5'-CCG AGC GAA TGT AGG AGT CCA GGG TCT CTG CTC GG	153,880,2138[b,c]	280-302[d]
Bacterial species:	***Staphylococcus aureus***		
2186	5'-ACG CGC TCA AAG CAG AAG TAT ACG TAT TAT CAA AAG ACG CGC GT	1728	615-646
Bacterial group:	***Staphylococcus* sp. other than *S. aureus***		
1233	5'-GCG AGC GTT ACT GGT GTA GAA ATG TTC CGG CTC GC	878	372-394
Fungal species:	***Candida albicans***		
2073	5'-CCG AGC AAC ATG ATT GAA CCA TCC ACC AAC TGG CTC GG	408	404-429
Fungal species:	***Candida dubliniensis***		
2074	5'-CCG AGC AAC ATG ATT GAA GCT TCC ACC AAC TGG CTC GG	414	416-441
Fungal species:	***Candida glabrata***		
2110[b]	5'-GCG GGC CCT TAA CGA TTT CAG CGA ATC TGG ATT CAG CCC GC	417	307-335
2111	5'-GCG GGC ATG TTG AAG CCA CCA CCA ACG CTT CCT GGC CCG C	417	419-447
Fungal species:	***Candida krusei***		
2112[b]	5'-GCG GGC TTG ATG AAG TTT GGG TTT CCT TGA CAA TTG CCC GC	422	318-347
2113	5'-GCG GGC ACA AGG GTT GGA CTA AGG AAA CCA AGG CAG CCC GC	422	419-447
2114	5'-GCG GGC ATC GAT GCT ATT GAA CCA CCT GTC AGA CCG CCC GC	422	505-533

a Underlined nucleotides indicate the molecular beacon's stem.

b Sequence from databases.

c These sequences were aligned to derive the corresponding primer.

d The nucleotide positions refer to the *P. aeruginosa tuf* sequence fragment (SEQ ID NO. 153).

Annex XLVII: Molecular beacon internal hybridization prob s for specific d tection of *tuf* sequences (continued).

SEQ ID NO.	Nucleotide sequence[a]	Originating DNA fragment SEQ ID NO.	Nucleotide position
Fungal species:	***Candida lusitaniae***		
2115[b]	5'-GCG GGC GGT AAG TCC ACC GGT AAG ACC TTG TTG GCC CGC	424	304-330
2116	5'-GCG GGC GTA AGT CAC CGG TAA GAC CTT GTT GGC CCG C	424	476-502
2117	5'-GCG GGC GAC GCC ATT GAG CCA CCT TCG AGA GCC CGC	424	512-535
Fungal species:	***Candida parapsilosis***		
2118[b]	5'-GCG GGC TCC TTG ACA ATT TCT TCG TAT CTG TTC TTG GCC CGC	426	301-330
Fungal species:	***Candida tropicalis***		
2119	5'-GCG GGC TTA CAA CCC TAA GGC TGT TCC ATT CGT TGC CCG C	429	357-384
2120	5'-GCG GGC AGA AAC CAA GGC TGG TAA GGT TAC CGG AGC CCG C	429	459-487
Fungal species:	***Cryptococcus neoformans***		
2106	5'-GCG AGC AGA GCA CGC CCT CCT CGC CGC TCG C	623,1985,1986[c]	226-244[d]
2107	5'-GCG AGC TCC CCA TCT CTG GTT GGC ACG CTC GC	623,1985,1986[c]	390-408[d]
Bacterial genus:	***Legionella sp.***		
2083	5'-CCG CCG ATG TTC CGT AAA TTA CTT GAI GAA GGT CGA GCC GGC GG	111-112[a]	488-519[e]

a Underlined nucleotides indicate the molecular beacon's stem.

b This sequence is from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

c These sequences were aligned to derive the corresponding primer.

d The nucleotide positions refer to the *C. neoformans tuf* (EF-1) sequence fragment (SEQ ID NO. 623).

e The nucleotide positions refer to the *L. pneumophila tuf* (EF-1) sequence fragment (SEQ ID NO. 112).

Annex XLVII: Molecular beacon internal hybridization probes for specific detection of *tuf* sequences (continued).

SEQ ID NO.	Nucleotide sequence[a]	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Fungal genus:	***Candida sp.***		
2108	5'-GCG GGC AAC TTC RTC AAG AAG GTT GGT TAC AAC CCG CCC GC	414,417, 422,424, 426,429,624[b]	52-80[c]
2109	5'-GCG GGC CCA ATC TCT GGT TGG AAY GGT GAC AAG CCC GC	Same as SEQ ID NO. 2108	100-125[c]
Bacterial group:	**Pseudomonads**		
2121	5'-CGA CCG CIA GCC GCA CAC CAA GTT CCG GTC G	153-155, 205,880,2137[d], 2138[d,b]	598-616[e]

[a] Underlined nucleotides indicate the molecular beacon's stem.
[b] These sequences were aligned to derive the corresponding primer.
[c] The nucleotide positions refer to the *C. albicans tuf* (EF-1) sequence fragment (SEQ ID NO. 624).
[d] Sequence from databases.
[e] The nucleotide positions refer to the *P. aeruginosa tuf* sequence fragment (SEQ ID NO. 153).

Annex XLVIII: Molecular beacon internal hybridization prob s for specific detection of *ddl* and *mtl* gene sequences.

SEQ ID NO.	Nucleotide sequence[a]	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Bacterial species:	***E. faecium (ddl)***		
1237	5'-GCG AGC CGC GAA ATC GAA GTT GCT GTA TTA GGG CTC GC	1242[b]	334-359
Bacterial species:	***E. faecalis (mtl)***		
1238	5'-GCG AGC GGC GTT AAT TTT GGC ACC GAA GAA GAG CTC GC	1243[b]	631-656

[a] Underlined nucleotides indicate the molecular beacon's stem.
[b] Sequence from databases.

Annex XLIX: Internal hybridization probe for specific detection of *S.* aur us sequenc s of unknown coding potential.

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment	
		SEQ ID NO.	Nucleotide position
Bacterial species:	***Staphylococcus aureus***		
1234	5'-ACT AAA TAA ACG CTC ATT CG	1244	35-54

Annex L: Specific and ubiquitous primers for nucleic acid amplification (antimicrobial ag nts resistance genes sequences).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Resistance gene:	***aac(2')-Ia***		
1344	5'-AGC AGC AAC GAT GTT ACG CAG CAG	1348[a]	163-186
1345[b]	5'-CCC GCC GAG CAT TTC AAC TAT TG	1348[a]	392-414
1346	5'-GAT GTT ACG CAG CAG GGC AGT C	1348[a]	172-193
1347[b]	5'-ACC AAG CAG GTT CGC AGT CAA GTA	1348[a]	467-490
Resistance gene:	***aac(3')-Ib***		
1349	5'-CAG CCG ACC AAT GAG TAT CTT GCC	1351[a]	178-201
1350[b]	5'-TAA TCA GGG CAG TTG CGA CTC CTA	1351[a]	356-379
Resistance gene:	***aac(3')-IIb***		
1352	5'-CCA CGC TGA CAG AGC CGC ACC G	1356[a]	383-404
1353[b]	5'-GGC CAG CTC CCA TCG GAC CCT G	1356[a]	585-606
1354	5'-CAC GCT GAC AGA GCC GCA CCG	1356[a]	384-404
1355[b]	5'-ATG CCG TTG CTG TCG AAA TCC TCG	1356[a]	606-629
Resistance gene:	***aac(3')-IVa***		
1357	5'-GCC CAT CCA TTT GCC TTT GC	1361[a]	295-314
1358[b]	5'-GCG TAC CAA CTT GCC ATC CTG AAG	1361[a]	517-540
1359	5'-TGC CCC TGC CAC CTC ACT C	1361[a]	356-374
1360[b]	5'-CGT ACC AAC TTG CCA TCC TGA AGA	1361[a]	516-539
Resistance gene:	***aac(3')-VIa***		
1362	5'-CGC CGC CAT CGC CCA AAG CTG G	1366[a]	285-306
1363[b]	5'-CGG CAT AAT GGA GCG CGG TGA CTG	1366[a]	551-574
1364	5'-TTT CTC GCC CAC GCA GGA AAA ATC	1366[a]	502-525
1365[b]	5'-CAT CCT CGA CGA ATA TGC CGC G	1366[a]	681-702
Resistance gene:	***aac(6')-Ia***		
1367	5'-CAA ATA TAC TAA CAG AAG CGT TCA	1371[a]	56-79
1368[b]	5'-AGG ATC TTG CCA ATA CCT TTA T	1371[a]	269-290
1379	5'-AAA CCT TTG TTT CGG TCT GCT AAT	1371[a]	153-176
1380[b]	5'-AAG CGA TTC CAA TAA TAC CTT GCT	1371[a]	320-343

[a] Sequence from databases.

[b] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex L: Specific and ubiquitous primers for nucleic acid amplification (antimicrobial agents resistance genes sequences) (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Resistance gene:	***aac(6')-Ic***		
1372	5'-GCT TTC GTT GCC TTT GCC GAG GTC	1376[a]	157-180
1373[b]	5'-CAC CCC TGT TGC TTC GCC CAC TC	1376[a]	304-326
1374	5'-AGA TAT TGG CTT CGC CGC ACC ACA	1376[a]	104-127
1375[b]	5'-CCC TGT TGC TTC GCC CAC TCC TG	1376[a]	301-323
Resistance gene:	***ant(3')-Ia***		
1377	5'-GCC GTG GGT CGA TGT TTG ATG TTA	1381[a]	100-123
1378[b]	5'-GCT CGA TGA CGC CAA CTA CCT CTG	1381[a]	221-244
1379	5'-AGC AGC AAC GAT GTT ACG CAG CAG	1381[a]	127-150
1380[b]	5'-CGC TCG ATG ACG CCA ACT ACC TCT	1381[a]	222-245
Resistance gene:	***ant(4')-Ia***		
1382	5'-TAG ATA TGA TAG GCG GTA AAA AGC	1386[a]	149-172
1383[b]	5'-CCC AAA TTC GAG TAA GAG GTA TT	1386[a]	386-408
1384	5'-GAT ATG ATA GGC GGT AAA AAG C	1386[a]	151-172
1385[b]	5'-TCC CAA ATT CGA GTA AGA GGT A	1386[a]	388-409
Resistance gene:	***aph(3')-Ia***		
1387	5'-TTA TGC CTC TTC CGA CCA TCA AGC	1391[a]	233-256
1338[b]	5'-TAC GCT CGT CAT CAA AAT CAC TCG	1391[a]	488-511
1389	5'-GAA TAA CGG TTT GGT TGA TGC GAG	1391[a]	468-491
1390[b]	5'-ATG GCA AGA TCC TGG TAT CGG TCT	1391[a]	669-692
Resistance gene:	***aph(3')-IIa***		
1392	5'-TGG GTG GAG AGG CTA TTC GGC TAT	1396[a]	43-66
1393[b]	5'-CAG TCC CTT CCC GCT TCA GTG AC	1396[a]	250-272
1394	5'-GAC GTT GTC ACT GAA GCG GGA AGG	1396[a]	244-267
1395[b]	5'-CTT GGT GGT CGA ATG GGC AGG TAG	1396[a]	386-409

[a] Sequence from databases.

[b] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex L: Specific and ubiquitous primers for nucleic acid amplification (antimicrobial agents resistance genes sequences) (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Resistance gene:	***aph(3')-IIIa***		
1397	5'-GTG GGA GAA AAT GAA AAC CTA T	1401[a]	103-124
1398[b]	5'-ATG GAG TGA AAG AGC CTG AT	1401[a]	355-374
1399	5'-ACC TAT GAT GTG GAA CGG GAA AAG	1401[a]	160-183
1400[b]	5'-CGA TGG AGT GAA AGA GCC TGA TG	1401[a]	354-376
Resistance gene:	***aph(3')-VIa***		
1402	5'-TAT TCA ACA ATT TAT CGG AAA CAG	1406[a]	18-41
1403[b]	5'-TCA GAG AGC CAA CTC AAC ATT TT	1406[a]	175-197
1404	5'-AAA CAG CGT TTT AGA GCC AAA TAA	1406[a]	36-59
1405[b]	5'-TTC TCA GAG AGC CAA CTC AAC ATT	1406[a]	177-200
Resistance gene:	***blaCARB***		
1407	5'-CCC TGT AAT AGA AAA GCA AGT AGG	1411[a]	351-374
1408[b]	5'-TTG TCG TAT CCC TCA AAT CAC C	1411[a]	556-577
1409	5'-TGG GAT TAC AAT GGC AAT CAG CG	1411[a]	205-227
1410[b]	5'-GGG GAA TAG GTC ACA AGA TCT GCT T	1411[a]	329-353
Resistance gene:	***blaCMY-2***		
1412	5'-GAG AAA ACG CTC CAG CAG GGC	1416[a]	793-813
1413[b]	5'-CAT GAG GCT TTC ACT GCG GGG	1416[a]	975-995
1414	5'-TAT CGT TAA TCG CAC CAT CAC	1416[a]	90-110
1415[b]	5'-ATG CAG TAA TGC GGC TTT ATC	1416[a]	439-459
Resistance genes:	***blaCTX-M-1, blaCTX-M-2***		
1417	5'-TGG TTA ACT AYA ATC CSA TTG CGG A	1423[a]	314-338
1418[b]	5'-ATG CTT TAC CCA GCG TCA GAT T	1423[a]	583-604
Resistance gene:	***blaCTX-M-1***		
1419	5'-CGA TGA ATA AGC TGA TTT CTC ACG	1423[a]	410-433
1420[b]	5'-TGC TTT ACC CAG CGT CAG ATT ACG	1423[a]	580-603
1421	5'-AAT TAG AGC GGC AGT CGG GAG GAA	1423[a]	116-139
1422[b]	5'-GAA ATC AGC TTA TTC ATC GCC ACG	1423[a]	405-428

a Sequence from databases.

b These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex L: Specific and ubiquitous primers for nucl ic acid amplification (antimicrobial agents resistance genes sequences) (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Nucleotide position
Resistance gene:	***blaCTX-M-2***		
1424	5'-GTT AAC GGT GAT GGC GAC GCT AC	1428[a]	30-52
1425[b]	5'-GAA TTA TCG GCG GTG TTA ATC AGC	1428[a]	153-176
1426	5'-CAC GCT CAA TAC CGC CAT TCC A	1428[a]	510-531
1427[b]	5'-TTA TCG CCC ACT ACC CAT GAT TTC	1428[a]	687-710
Resistance gene:	***blaIMP***		
1429	5'-TTT ACG GCT AAA GAT ACT GAA AAG T	1433[a]	205-229
1430[b]	5'-GTT TAA TAA AAC AAC CAC CGA ATA AT	1433[a]	513-538
1431	5'-TAA TTG ACA CTC CAT TTA CGG CTA A	1433[a]	191-215
1432[b]	5'-ACC GAA TAA TAT TTT CCT TTC AGG CA	1433[a]	497-522
Resistance gene:	***blaOXA2***		
1434	5'-CAC AAT CAA GAC CAA GAT TTG CGA T	1438[a]	319-343
1435[b]	5'-GAA AGG GCA GCT CGT TAC GAT AGA G	1438[a]	532-556
Resistance gene:	***blaOXA10***		
1436	5'-CAG CAT CAA CAT TTA AGA TCC CCA	1439[a]	194-217
1437[b]	5'-CTC CAC TTG ATT AAC TGC GGA AAT TC	1439[a]	479-504
Resistance gene:	***blaPER-1***		
1440	5'-AGA CCG TTA TCG TAA ACA GGG CTA AG	1442[a]	281-306
1441[b]	5'-TTT TTT GCT CAA ACT TTT TCA GGA TC	1442[a]	579-604
Resistance gene:	***blaPER-2***		
1443	5'-CTT CTG CTC TGC TGA TGC TTG GC	1445[a]	32-54
1444[b]	5'-GGC GAC CAG GTA TTT TGT AAT ACT GC	1445[a]	304-329
Resistance genes:	***blaPER-1, blaPER-2***		
1446	5'-GGC CTG YGA TTT GTT ATT TGA ACT GGT	1442[a]	414-440
1447[b]	5'-CGC TST GGT CCT GTG GTG GTT TC	1442[a]	652-674
1448	5'-GAT CAG GTG CAR TAT CAA AAC TGG AC	1442[a]	532-557
1449[b]	5'-AGC WGG TAA CAA YCC TTT TAA CCG CT	1442[a]	671-696

[a] Sequence from databases.

[b] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex L: Specific and ubiquitous primers for nucleic acid amplification (antimicrobial agents resistance genes sequences) (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Resistance gene:	***blaSHV***		
1883	5'-AGC CGC TTG AGC AAA TTA AAC TA	1900[a]	71-93
1884[b]	5'-GTA TCC CGC AGA TAA ATC ACC AC	1900[a]	763-785
1885	5'-AGC GAA AAA CAC CTT GCC GAC	1900[a]	313-333
1884[b]	5'-GTA TCC CGC AGA TAA ATC ACC AC	1900[a]	763-785
Resistance gene:	***blaTEM***		
1906	5'-CCT TAT TCC CTT TTT TGC GG	1927[a]	27-46
1907[b]	5'-CAC CTA TCT CAG CGA TCT GTC T	1927[a]	817-838
1908	5'-AAC AGC GGT AAG ATC CTT GAG AG	1927[a]	148-170
1907[b]	5'-CAC CTA TCT CAG CGA TCT GTC T	1927[a]	817-838
Resistance gene:	***catI***		
2145	5'-GCA AGA TGT GGC GTG TTA CGG T	2147[a]	363-384
2146[b]	5'-GGG GCG AAG AAG TTG TCC ATA TT	2147[a]	484-506
Resistance gene:	***catII***		
2148	5'-CAG ATT AAA TGC GGA TTC AGC C	2150[a]	67-88
2149[b]	5'-ATC AGG TAA ATC ATC AGC GGA TA	2150[a]	151-173
Resistance gene:	***catIII***		
2151	5'-ATA TTT CAG CAT TAC CTT GGG TT	2153[a]	419-441
2152[b]	5'-TAC ACA ACT CTT GTA GCC GAT TA	2153[a]	603-625
Resistance gene:	***catP***		
2154	5'-CGC CAT TCA GAG TTT AGG AC	2156[a]	178-197
2155[b]	5'-TTC CAT ACC GTT GCG TAT CAC TT	2156[a]	339-361
Resistance gene:	***cat***		
2157	5'-CCA CAG AAA TTG ATA TTA GTG TTT TAT	2159[a]	89-115
2158[b]	5'-TCG CTA TTG TAA CCA GTT CTA	2159[a]	201-221
2160	5'-TTT TGA ACA CTA TTT TAA CCA GC	2162[a]	48-70
2161[b]	5'-GAT TTA ACT TAT CCC AAT AAC CT	2162[a]	231-253

[a] Sequence from databases.

[b] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex L: Specific and ubiquitous primers for nucleic acid amplification (antimicrobial agents resistance genes sequences) (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Resistance gene:	***dfrA***		
1450	5'-ACC ACT GGG AAT ACA CTT GTA ATG GC	1452[a]	106-131
1451[b]	5'-ATC TAC CTG GTC AAT CAT TGC TTC GT	1452[a]	296-321
Resistance gene:	***dhfrIa***		
1457	5'-CAA AGG TGA ACA GCT CCT GTT T	1461[a]	75-96
1458[b]	5'-TCC GTT ATT TTC TTT AGG TTG GTT AAA	1461[a]	249-275
1459	5'-AAG GTG AAC AGC TCC TGT TT	1461[a]	77-96
1560[b]	5'-GAT CAC TAC GTT CTC ATT GTC A	1461[a]	207-228
Resistance genes:	***dhfrIa, dhfrXV***		
1453	5'-ATC GAA GAA TGG AGT TAT CGG RAA TG	1461[a]	27-52
1454[b]	5'-CCT AAA AYT RCT GGG GAT TTC WGG A	1461[a]	384-408
1455	5'-CAG GTG GTG GGG AGA TAT ACA AAA	1461[a]	290-313
1456[b]	5'-TAT GTT AGA SRC GAA GTC TTG GKT AA	1461[a]	416-441
Resistance gene:	***dhfrIb***		
1466	5'-AAG CAT TGA CCT ACA ATC AGT GT	1470[a]	98-120
1467[b]	5'-AAT ACA ACT ACA TTG TCA TCA TTT GAT	1470[a]	204-230
1468	5'-CGT TAC CCG CTC AGG TTG GAC ATC AA	1470[a]	183-208
1469[b]	5'-CAT CCC CCT CTG GCT CGA TGT CG	1470[a]	354-376
Resistance gene:	***dhfrV***		
1471	5'-GAT AAT GAC AAC GTA ATA GTA TTC CC	1475[a]	208-233
1472[b]	5'-GCT CAA TAT CAA TCG TCG ATA TA	1475[a]	342-364
1473	5'-TTA AAG CCT TGA CGT ACA ACC AGT GG	1475[a]	95-120
1474[b]	5'-TGG GCA ATG TTT CTC TGT AAA TCT CC	1475[a]	300-325
Resistance genes:	***dhfrIb, dhfrV***		
1462	5'-GCA CTC CCY AAT AGG AAA TAC GC	1470[a]	157-179
1463[b]	5'-AGT GTT GCT CAA AAA CAA CTT CG	1470[a]	405-427
1464	5'-ACG TTY GAA TCT ATG GGM GCA CT	1470[a]	139-161
1465[b]	5'-GTC GAT AAG TGG AGC GTA GAG GC	1470[a]	328-350

[a] Sequence from databases.

[b] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex L: Specific and ubiquitous primers for nucleic acid amplification (antimicrobial agents r sistanc genes sequences) (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Nucleotide position
Resistance gene:	***dhfrVI***		
1476	5'-GGC GAG CAG CTC CTA TTC AAA G	1480[a]	79-100
1477[b]	5'-TAG GTA AGC TAA TGC CGA TTC AAC A	1480[a]	237-261
1478	5'-GAG AAT GGA GTA ATT GGC TCT GGA TT	1480[a]	31-56
1479[b]	5'-GCG AAA TAC ACA ACA TCA GGG TCA T	1480[a]	209-233
Resistance gene:	***dhfrVII***		
1485	5'-AAA ATG GCG TAA TCG GTA ATG GC	1489[a]	32-54
1486[b]	5'-CAT TTG AGC TTG AAA TTC CTT TCC TC	1489[a]	189-214
1487	5'-AAT CGA AAA TAT GCA GTA GTG TCG AG	1489[a]	166-191
1488[b]	5'-AGA CTA TTG TAG ATT TGA CCG CCA	1489[a]	294-317
Resistance genes:	***dhfrVII, dhfrXVII***		
1481	5'-RTT ACA GAT CAT KTA TAT GTC TCT	1489[a]	268-291
1482[b]	5'-TAA TTT ATA TTA GAC AWA AAA AAC TG	1489[a]	421-446
1483	5'-CAR YGT CAG AAA ATG GCG TAA TC	1489[a]	23-45
1484[b]	5'-TKC AAA GCR WTT TCT ATT GAA GGA AA	1489[a]	229-254
Resistance gene:	***dhfrVIII***		
1490	5'-GAC CTA TGA GAG CTT GCC CGT CAA A	1494[a]	144-168
1491[b]	5'-TCG CCT TCG TAC AGT CGC TTA ACA AA	1494[a]	376-401
1492	5'-CAT TTT AGC TGC CAC CGC CAA TGG TT	1494[a]	18-43
1493[b]	5'-GCG TCG CTG ACG TTG TTC ACG AAG A	1494[a]	245-269
Resistance gene:	***dhfrIX***		
1495	5'-TCT CTA AAC ATG ATT GTC GCT GTC	1499[a]	7-30
1496[b]	5'-CAG TGA GGC AAA AGT TTT TCT ACC	1499[a]	133-156
1497	5'-CGG ACG ACT TCA TGT GGT AGT CAG T	1499[a]	171-195
1498[b]	5'-TTT GTT TTC AGT AAT GGT CGG GAC CT	1499[a]	446-471

a Sequence from databases.
b These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex L: Specific and ubiquitous primers for nucleic acid amplification (antimicrobial agents resistance genes sequences) (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Resistance gene:	***dhfrXII***		
1500	5'-ATC GGG TTA TTG GCA ATG GTC CTA	1504[a]	50-73
1501[b]	5'-GCG GTA GTT AGC TTG GCG TGA GAT T	1504[a]	201-225
1502	5'-GCG GGC GGA GCT GAG ATA TAC A	1504[a]	304-325
1503[b]	5'-AAC GGA GTG GGT GTA CGG AAT TAC AG	1504[a]	452-477
Resistance gene:	***dhfrXIII***		
1505	5'-ATT TTT CGC AGG CTC ACC GAG AGC	1507[a]	106-129
1506[b]	5'-CGG ATG AGA CAA CCT CGA ATT CTG CTG	1507[a]	413-439
Resistance gene:	***dhfrXV***		
1508	5'-AGA ATG TAT TGG TAT TTC CAT CTA TCG	1512[a]	215-241
1509[b]	5'-CAA TGT CGA TTG TTG AAA TAT GTA AA	1512[a]	336-361
1510	5'-TGG AGT GCC AAA GGG GAA CAA T	1512[a]	67-88
1511[b]	5'-CAG ACA CAA TCA CAT GAT CCG TTA TCG	1512[a]	266-292
Resistance gene:	***dhfrXVII***		
1513	5'-TTC AAG CTC AAA TGA AAA CGT CC	1517[a]	201-223
1514[b]	5'-GAA ATT CTC AGG CAT TAT AGG GAA T	1517[a]	381-405
1515	5'-GTG GTC AGT AAA AGG TGA GCA AC	1517[a]	66-88
1516[b]	5'-TCT TTC AAA GCA TTT TCT ATT GAA GG	1517[a]	232-257
Resistance gene:	***embB***		
2102	5'-CAC CTT CAC CCT GAC CGA CG	2105[a]	822-841
2103[b]	5'-CGA ACC AGC GGA AAT AGT TGG AC	2105[a]	948-970
Resistance genes:	***ereA, ereA2***		
1528	5'-AAC TTG AGC GAT TTT CGG ATA CCC TG	1530[a]	80-105
1529[b]	5'-TTG CCG ATG AAA TAA CCG CCG ACT	1530[a]	317-340

[a] Sequence from databases.

[b] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex L: Specific and ubiquitous primers for nucl ic acid amplification (antimicrobial agents resistance genes sequences) (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Resistance gene:	***ereB***		
1531	5'-TCT TTT TGT TAC GAC ATA CGC TTT T	1535[a]	152-176
1532[b]	5'-AGT GCT TCT TTA TCC GCT GTT CTA	1535[a]	456-479
1533	5'-CAG CGG ATA AAG AAG CAC TAC ACA TT	1535[a]	461-486
1534[b]	5'-CCT CCT GAA ATA AAG CCC GAC AT	1535[a]	727-749
Resistance gene:	***gyrA***		
1340	5'-GAA CAA GGT ATG ACA CCG GAT AAA T	1299[a]	163-188
1341[b]	5'-GAT AAC TGA AAT CCT GAG CCA TAC G	1299[a]	274-299
1936	5'-TAC CAC CCG CAC GGC	1954[a]	205-219
1937[b]	5'-CGG AGT CGC CGT CGA TG	1954[a]	309-325
1942	5'-GAC TGG AAC AAA GCC TAT AAA AAA TCA	1954[a]	148-174
1937[b]	5'-CGG AGT CGC CGT CGA TG	1954[a]	309-325
2040	5'-TGT GAC CCC AGA CAA ACC C	2054[a]	33-51
2041[b]	5'-GTT GAG CGG CAG CAC TAT CT	2054[a]	207-226
Resistance gene:	***inhA***		
2098	5'-CTG AGT CAC ACC GAC AAA CGT C	2101[a]	910-931
2099[b]	5'-CCA GGA CTG AAC GGG ATA CGA A	2101[a]	1074-1095
Resistance genes:	***linA, linA'***		
1536	5'-AGA TGT ATT AAC TGG AAA ACA ACA A	1540[a]	99-123
1537[b]	5'-CTT TGT AAT TAG TTT CTG AAA ACC A	1540[a]	352-376
1538	5'-TTA GAA GAT ATA GGA TAC AAA ATA GAA G	1540[a]	187-214
1539[b]	5'-GAA TGA AAA AGA AGT TGA GCT T	1540[a]	404-425
Resistance gene:	***linB***		
1541	5'-TGA TAA TCT TAT ACG TGG GGA ATT T	1545[a]	246-270
1542[b]	5'-ATA ATT TTC TAA TTG CCC TGT TTC AT	1545[a]	359-384
1543	5'-GGG CAA TTA GAA AAT TAT TTA TCA GA	1545[a]	367-392
1544[b]	5'-TTT TAC TCA TGT TTA GCC AAT TAT CA	1545[a]	579-604

[a] Sequence from databases.

[b] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex L: Specific and ubiquitous primers for nucleic acid amplification (antimicrobial agents resistance genes sequences) (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Resistance gene:	***mefA***		
1546	5'-CAA GAA GGA ATG GCT GTA CTA C	1548[a]	625-646
1547[b]	5'-TAA TTC CCA AAT AAC CCT AAT AAT AGA	1548[a]	816-842
Resistance gene:	***mefE***		
1549	5'-GCT TAT TAT TAG GAA GAT TAG GGG GC	1551[a]	815-840
1550[b]	5'-TAG CAA GTG ACA TGA TAC TTC CGA	1551[a]	1052-1075
Resistance genes:	***mefA, mefE***		
1552	5'-GGC AAG CAG TAT CAT TAA TCA CTA	1548[a]	50-73
1553[b]	5'-CAA TGC TAC GGA TAA ACA ATA CTA TC	1548[a]	318-343
1554	5'-AGA AAA TTA AGC CTG AAT ATT TAG GAC	1548[a]	1010-1035
1555[b]	5'-TAG TAA AAA CCA ATG ATT TAC ACC G	1548[a]	1119-1143
Resistance genes:	***mphA, mphK***		
1556	5'-ACT GTA CGC ACT TGC AGC CCG ACA T	1560[a]	33-57
1557[b]	5'-GAA CGG CAG GCG ATT CTT GAG CAT	1560[a]	214-237
1558	5'-GTG GTG GTG CAT GGC GAT CTC T	1560[a]	583-604
1559[b]	5'-GCC GCA GCG AGG TAC TCT TCG TTA	1560[a]	855-878
Resistance gene:	***mupA***		
2142	5'-GCC TTA ATT TCG GAT AGT GC	2144[a]	1831-1850
2143[b]	5'-GAG AAA GAG CCC AAT TAT CTA ATG T	2144[a]	2002-2026
Resistance gene:	***parC***		
1342	5'-GAT GTT ATT GGT CAA TAT CAT CCA	1321[a]	205-229
1343[b]	5'-AAG AAA CTG TCT CTT TAT TAA TAT CAC GT	1321[a]	396-425
1934	5'-GAA CGC CAG CGC GAA ATT CAA AAA G	1781	67-91
1935[b]	5'-AGC TCG GCA TAC TTC GAC AGG	1781	277-297
2044	5'-ACC GTA AGT CGG CCA AGT CA	2055[a]	176-195
2045[b]	5'-GTT CTT TCT CCG TAT CGT C	2055[a]	436-454

a Sequence from databases.

b These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex L: Specific and ubiquitous primers for nucleic acid amplification (antimicrobial agents resistance genes sequences) (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Resistance gene:	***ppflo*-like**		
2163	5'-ACC TTC ATC CTA CCG ATG TGG GTT	2165[a]	922-945
2164[b]	5'-CAA CGA CAC CAG CAC TGC CAT TG	2165[a]	1136-1158
Resistance gene:	***rpoB***		
2065	5'-CCA GGA CGT GGA GGC GAT CAC A	2072[a]	1218-1239
2066[b]	5'-CAC CGA CAG CGA GCC GAT CAG A	2072[a]	1485-1506
Resistance gene:	***satG***		
1581	5'-AAT TGG GGA CTA CAC CTA TTA TGA TG	1585[a]	93-118
1582[b]	5'-GGC AAA TCA GTC AGT TCA GGA GT	1585[a]	310-332
1583	5'-CGA TTG GCA ACA ATA CAC TCC TG	1585[a]	294-316
1584[b]	5'-TCA CCT ATT TTT ACG CCT GGT AGG AC	1585[a]	388-413
Resistance gene:	***sulII***		
1961	5'-GCT CAA GGC AGA TGG CAT TCC C	1965[a]	222-243
1962[b]	5'-GGA CAA GGC GGT TGC GTT TGA T	1965[a]	496-517
1963	5'-CAT TCC CGT CTC GCT CGA CAG T	1965[a]	237-258
1964[b]	5'-ATC TGC CTG CCC GTC TTG C	1965[a]	393-411
Resistance gene:	***tetB***		
1966	5'-CAT GCC AGT CTT GCC AAC G	1970[a]	66-84
1967[b]	5'-CAG CAA TAA GTA ATC CAG CGA TG	1970[a]	242-264
1968	5'-GGA GAG ATT TCA CCG CAT AG	1970[a]	457-476
1969[b]	5'-AGC CAA CCA TCA TGC TAT TCC A	1970[a]	721-742
Resistance gene:	***tetM***		
1586	5'-ATT CCC ACA ATC TTT TTT ATC AAT AA	1590[a]	361-386
1587[b]	5'-CAT TGT TCA GAT TCG GTA AAG TTC	1590[a]	501-524
1588	5'-GTT TTT GAA GTT AAA TAG TGT TCT T	1590[a]	957-981
1589[b]	5'-CTT CCA TTT GTA CTT TCC CTA	1590[a]	1172-1192

[a] Sequence from databases.
[b] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex L: **Specific and ubiquitous primers for nucleic acid amplification (antimicrobial agents resistance genes s quences) (continued).**

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Resistance gene:	***vatB***		
1609	5'-GCC CTG ATC CAA ATA GCA TAT A	1613[a]	11-32
1610[b]	5'-CCT GGC ATA ACA GTA ACA TTC TG	1613[a]	379-401
1611	5'-TGG GAA AAA GCA ACT CCA TCT C	1613[a]	301-322
1612[b]	5'-ACA ACT GAA TTC GCA GCA ACA AT	1613[a]	424-446
Resistance gene:	***vatC***		
1614	5'-CCA ATC CAG AAG AAA TAT ACC C	1618[a]	26-47
1615[b]	5'-ATT AGT TTA TCC CCA ATC AAT TCA	1618[a]	177-200
1616	5'-ATA ATG AAT GGG GCT AAT CAT CGT AT	1618[a]	241-266
1617[b]	5'-GCC AAC AAC TGA ATA AGG ATC AAC	1618[a]	463-486
Resistance gene:	***vga***		
1619	5'-AAG GCA AAA TAA AAG GAG CAA AGC	1623[a]	641-664
1620[b]	5'-TGT ACC CGA GAC ATC TTC ACC AC	1623[a]	821-843
1621	5'-AAT TGA AGG ACG GGT ATT GTG GAA AG	1623[a]	843-868
1622[b]	5'-CGA TTT TGA CAG ATG GCG ATA ATG AA	1623[a]	975-1000
Resistance gene:	***vgaB***		
1624	5'-TTC TTT AAT GCT CGT AGA TGA ACC TA	1628[a]	354-379
1625[b]	5'-TTT TCG TAT TCT TCT TGT TGC TTT C	1628[a]	578-602
1626	5'-AGG AAT GAT TAA GCC CCC TTC AAA AA	1628[a]	663-688
1627[b]	5'-TTA CAT TGC GAC CAT GAA ATT GCT CT	1628[a]	849-874
Resistance genes:	***vgb, vgh***		
1629	5'-AAG GGG AAA GTT TGG ATT ACA CAA CA	1633[a]	73-98
1630[b]	5'-GAA CCA CAG GGC ATT ATC AGA ACC	1633[a]	445-468
1631	5'-CGA CGA TGC TTT ATG GTT TGT	1633[a]	576-596
1632[b]	5'-GTT AAT TTG CCT ATC TTG TCA CAC TC	1633[a]	850-875

a Sequence from databases.
b These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex L: Specific and ubiquitous primers for nucleic acid amplification (antimicrobial agents resistance genes sequences) (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Resistance gene:	***vgbB***		
1634	5'-TTA ACT TGT CTA TTC CCG ATT CAG G	1882[a]	23-47
1635[b]	5'-GCT GTG GCA ATG GAT ATT CTG TA	1882[a]	267-289
1636	5'-TTC CTA CCC CTG ATG CTA AAG TGA	1882[a]	155-178
1637[b]	5'-CAA AGT GCG TTA TCC GAA CCT AA	1882[a]	442-464
	Sequencing primers		
Resistance gene:	***gyrA***		
1290	5'-GAY TAY GCI ATG ISI GTI ATH GT	1299[a]	70-83
1292[b]	5'-ARI SCY TCI ARI ATR TGI GC	1299[a]	1132-1152
1291	5'-GCI YTI CCI GAY GTI MGI GAY GG	1299[a]	100-123
1292[b]	5'-ARI SCY TCI ARI ATR TGI GC	1299[a]	1132-1152
1293	5'-ATG GCT GAA TTA CCT CAA TC	1299[a]	1-21
1294[b]	5'-ATG ATT GTT GTA TAT CTT CTT CAA C	1299[a]	2626-2651
1295[b]	5'-CAG AAA GTT TGA AGC GTT GT	1299[a]	1255-1275
1296	5'-AAC GAT TCG TGA GTC AGA TA	1299[a]	1188-1208
1297	5'-CGG TCA ACA TTG AGG AAG AGC T	1300[a]	29-51
1298[b]	5'-ACG AAA TCG ACC GTC TCT TTT TC	1300[a]	415-437
Resistance gene:	***gyrB***		
1301	5'-GTI MGI AWI MGI CCI GSI ATG TA	1307[a]	82-105
1302[b]	5'-TAI ADI GGI GGI KKI GCI ATR TA	1307[a]	1600-1623
1303	5'-GGI GAI GAI DYI MGI GAR GG	1307[a]	955-975
1304[b]	5'-CIA RYT TIK YIT TIG TYT G	1307[a]	1024-1043
1305	5'-ATG GTG ACT GCA TTG TCA GAT G	1307[a]	1-23
1306[b]	5'-GTC TAC GGT TTT CTA CAA CGT C	1307[a]	1858-1888

[a] Sequence from databases.

[b] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex L: Specific and ubiquitous primers for nucleic acid amplification (antimicrobial agents resistance genes sequences) (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment: SEQ ID NO.	Originating DNA fragment: Nucleotide position
	Sequencing primers (continued)		
Resistance gene:	***parC***		
1308	5'-ATG TAY GTI ATI ATG GAY MGI GC	1320[a]	67-90
1309[b]	5'-ATI ATY TTR TTI CCY TTI CCY TT	1320[a]	1993-2016
1310	5'-ATI ATI TSI ATI ACY TCR TC	1320[a]	1112-1132
1311[b]	5'-GAR ATG AAR ATI MGI GGI GAR CA	1320[a]	1288-1311
1312	5'-AAR TAY ATI ATI CAR GAR MGI GC	1321[a]	67-90
1313[b]	5'-AMI AYI CKR TGI GGI TTI TTY TT	1321[a]	2212-2235
1314	5'-TAI GAI TTY ACI GAI SMI CAR GC	1321[a]	1228-1251
1315[b]	5'-ACI ATI GCI TCI GCY TGI KSY TC	1321[a]	1240-1263
1316	5'-GTG AGT GAA ATA ATT CAA GAT T	1321[a]	1-23
1317[b]	5'-CAC CAA AAT CAT CTG TAT CTA C	1321[a]	2356-2378
1318	5'-ACC TAY TCS ATG TAC GTR ATC ATG GA	1320[a]	58-84
1319[b]	5'-AGR TCG TCI ACC ATC GGY AGY TT	1320[a]	832-855
Resistance gene:	***parE***		
1322	5'-RTI GAI AAY ISI GTI GAY GAR G	1328[a]	133-155
1325[b]	5'-RTT CAT YTC ICC IAR ICC YTT	1328[a]	1732-1752
1323	5'-ACI AWR SAI GGI GGI ACI CAY G	1328[a]	829-850
1324[b]	5'-CCI CCI GCI SWR TCI CCY TC	1328[a]	1280-1302
1326	5'-TGA TTC AAT ACA GGT TTT AGA G	1328[a]	27-49
1327[b]	5'-CTA GAT TTC CTC CTC ATC AAA T	1328[a]	1971-1993

a Sequence from databases.

b These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex LI: Internal hybridization probes for specific det ction of antimicrobial agents resistance genes sequences.

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Resistance gene:	***aph3'VIa***		
2252	5'-CCA CAT ACA GTG TCT CTC	1406[a]	149-166
Resistance gene:	***blaSHV***		
1886	5'-GAC GCC CGC GCC ACC ACT	1900[a]	484-501
1887	5'-GAC GCC CGC GAC ACC ACT A	1899[a]	514-532
1888	5'-GAC GCC CGC AAC ACC ACT A	1901[a]	514-532
1889	5'-GTT CGC AAC TGC AGC TGC TG	1899[a]	593-612
1890	5'-TTC GCA ACG GCA GCT GCT G	1899[a]	594-612
1891	5'-CCG GAG CTG CCG AIC GGG	1902[a]	692-709
1892	5'-CGG AGC TGC CAA RCG GGG	1903[a]	693-710
1893	5'-GGA GCT GGC GAR CGG GGT	1899[a]	694-711
1894	5'-GAC CGG AGC TAG CGA RCG	1904[a]	690-707
1895	5'-CGG AGC TAG CAA RCG GGG T	1905[a]	693-711
1896	5'-GAA ACG GAA CTG AAT GAG GCG	1899[a]	484-504
1897	5'-CAT TAC CAT GGG CGA TAA CAG	1899[a]	366-386
1898	5'-CCA TTA CCA TGA GCG ATA ACAG	1899[a]	365-386
Resistance gene:	***blaTEM***		
1909	5'-ATG ACT TGG TTA AGT ACT CAC C	1928[a]	293-314
1910	5'-ATG ACT TGG TTG AGT ACT CAC C	1927[a]	293-314
1911	5'-CCA TAA CCA TGG GTG ATA ACA C	1928[a]	371-392
1912	5'-CCA TAA CCA TGA GTG ATA ACA C	1927[a]	371-392
1913	5'-CGC CTT GAT CAT TGG GAA CC	1928[a]	475-494
1914	5'-CGC CTT GAT CGT TGG GAA CC	1927[a]	475-494
1915	5'-CGC CTT GAT AGT TGG GAA CC	1929[a]	475-494
1916	5'-CGT GGG TCT TGC GGT ATC AT	1927[a]	712-731
1917	5'-CGT GGG TCT GGC GGT ATC AT	1930[a]	712-731
1918	5'-GTG GGT CTC ACG GTA TCA TTG	1927[a]	713-733
1919	5'-CGT GGG TCT CTC GGT ATC ATT	1931[a]	712-732
1920	5'-CGT GGI TCT CGC GGT ATC AT	1927[a]	712-731
1921	5'-CGT GGG TCT AGC GGT ATC ATT	1932[a]	713-733
1922	5'-GTT TTC CAA TGA TTA GCA CTT TTA	1927[a]	188-211
1923	5'-GTT TTC CAA TGA TAA GCA CTT TTA	1927[a]	188-211
1924	5'-GTT TTC CAA TGC TGA GCA CTT TT	1932[a]	188-210
1925	5'-CGT TTT CCA ATG ATG AGC ACT TT	1927[a]	187-209
1926	5'-GTT TTC CAA TGG TGA GCA CTT TT	1933[a]	188-210
2006	5'-TGG AGC CGG TGA GCG TGG	1927[a]	699-716

[a] Sequence from databases.

Annex LI: Internal hybridization probes for specific detection of antimicrobial agents resistance gen s sequences (continu d).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Resistance gene:	***blaTEM* (continued)**		
2007	5'-TGG AGC CAG TGA GCG TGG	2010[a]	699-716
2008	5'-TCT GGA GCC GAT GAG CGT G	1929[a]	697-715
2009	5'-CTG GAG CCA GTA AGC GTG G	2011[a]	698-716
2141	5'-CAC CAG TCA CAG AAA AGC	1927[a]	311-328
Resistance gene:	***dhfrIa***		
2253	5'-CAT TAC CCA ACC GAA AGT A	1461[a]	158-176
Resistance gene:	***embB***		
2104	5'-CTG GGC ATG GCI CGA GTC	2105[a]	910-927
Resistance gene:	***gyrA***		
1333	5'-TCA TGG TGA CTT ATC TAT TTA TG	1299[a]	240-263
1334	5'-CAT CTA TTT ATA AAG CAA TGG TA	1299[a]	251-274
1335	5'-CTA TTT ATG GAG CAA TGG T	1299[a]	254-273
1940	5'-GTA TCG TTG GTG ACG TAA T	1299[a]	206-224
1943	5'-GCT GGT GGA CGG CCA G	1954[a]	279-294
1945	5'-CGG CGA CTA CGC GGT AT	1954[a]	216-232
1946	5'-CGG CGA CTT CGC GGT AT	1954[a]	216-232
1947	5'-CGG TAT ACG GCA CCA TCG T	1954[a]	227-245
1948	5'-GCG GTA TAC AAC ACC ATC G	1954[a]	226-244
1949	5'-CGG TAT ACG CCA CCA TCG T	1954[a]	227-245
2042	5'-CAC GGG GAT TTC TCT ATT TA	2054[a]	103-122
2043	5'-CAC GGG GAT TAC TCT ATT TA	2054[a]	103-122
Resistance gene:	***inhA***		
2100	5'-GCG AGA CGA TAG GTT GTC	2101[a]	1017-1034
Resistance gene:	***parC***		
1336	5'-TGG AGA CTA CTC AGT GT	1321[a]	232-249
1337	5'-TGG AGA CTT CTC AGT GT	1321[a]	232-249
1338	5'-GTG TAC GGA GCA ATG	1321[a]	245-260
1339	5'-CCA GCG GAA ATG CGT	1321[a]	342-357
1941	5'-GCA ATG GTC CGT TTA AGT	1321[a]	253-270
1944	5'-TTT CGC CGC CAT GCG TTA C	1781	247-265
1950	5'-GGC GAC ATC GCC TGC	1781	137-151
1951	5'-GGC GAC AGA GCC TGC TA	1781	137-153

[a] Sequence from databases.

Annex LI: Internal hybridization probes for sp cific detection of antimicrobial ag nts resistance genes sequences (continued).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment	
		SEQ ID NO.	Nucleotide position
Resistance gene:	*parC* **(continued)**		
1952	5'-CCT GCT ATG GAG CGA TGG T	1781	147-165
1953	5'-CGC CTG CTA TAA AGC GAT GGT	1781	145-165
2046	5'-ACG GGG ATT TTT CTA TCT AT	2055[a]	227-246
Resistance gene:	***rpoB***		
2067	5'-AGC TGA GCC AAT TCA TGG	2072[a]	1304-1321
2068	5'-ATT CAT GGA CCA GAA CAA C	2072[a]	1314-1332
2069	5'-CGC TGT CGG GGT TGA CCC	2072[a]	1334-1351
2070	5'-GTT GAC CCA CAA GCG CCG	2072[a]	1344-1361
2071	5'-CGA CTG TCG GCG CTG GGG	2072[a]	1360-1377
Resistance gene:	***tetM***		
2254	5'-ACC TGA ACA GAG AGA AAT G	1590[a]	1062-1080

[a] Sequence from databases.

Annex LII: Molecular beacon internal hybridization probes for specific det ction of *atpD* sequences.

SEQ ID NO.	Nucleotide sequence[a]	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Bacterial species:	***Bacteroides fragilis***		
2136	5'-CCA ACG CGT CCT CAA TCA TTT CTA ACT TCT ATG GCC GGC GTT GG	929	353-382
Bacterial species:	***Bordetella pertussis***		
2182	5'-GCG CGC CAA CGA CTT CTA CCA CGA AAT GGA AGA GTC GCG CGC	1672	576-605
Bacterial group:	***Campylobacter jejuni* and *C. coli***		
2133	5'-CCA CGC ACA WAA ACT TGT TTT AGA AGT AGC AGC WCA GCG TGG	1576, 1600, 1849, 1863, 2139[b,c]	44-73[d]
Fungal species:	***Candida glabrata***		
2078	5'-CCG AGC CTT GGT CTT CGG CCA AAT GAA CGC TCG G	463	442-463
Fungal species:	***Candida krusei***		
2075	5'-CCG AGC CAG GTT CTG AAG TCT CTG CAT TAT TAG GTG CTC GG	468	720-748
Fungal species:	***Candida lusitaniae***		
2080	5'-CCG AGC CGA AGA GGG CCA AGA TGT CGC TCG G	470	520-538
Fungal species:	***Candida parapsilosis***		
2079	5'-CCG AGC GTT CAG TTA CTT CAG TCC AAG CCG GCT CGG	472	837-860
Fungal species:	***Candida tropicalis***		
2077	5'-CCG AGC AAC CGA TCC AGC TCC AGC TAC GCT CGG	475	877-897
Bacterial species:	***Klebsiella pneumoniae***		
2281	5'-CCC CCA GCT GGG CGG CGG TAT CGA TGG GGG	317	40-59

[a] Underlined nucleotides indicate the molecular beacon's stem.
[b] Sequence from databases.
[c] These sequences were aligned to derive the corresponding primer.
[d] The nucleotide positions refer to the *C. jejuni atpD* sequence fragment (SEQ ID NO. 1576).

Annex LII: Molecular beacon internal hybridization probes for specific detection of *atpD* sequences (continued).

SEQ ID NO.	Nucleotide sequence[a]	Originating DNA fragment	
		SEQ ID NO.	Nucleotide position
Fungal genus:	***Candida sp.***		
2076	5'-CCG AGC YGA YAA CAT TTT CAG ATT CAC CCA RGC GCT CGG	460-478, 663[b]	697-723[c]

[a] Underlined nucleotides indicate the molecular beacon's stem.
[b] These sequences were aligned to derive the corresponding primer.
[c] The nucleotide positions refer to the *C. albicans atpD* sequence fragment (SEQ ID NO. 460).

Annex LIII: Internal hybridization probes for sp cific detection of *atpD* sequences.

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment	
		SEQ ID NO.	Nucleotide position
Bacterial species:	***Acinetobacter baumannii***		
2169	5'-CCC GTT TGC GAA AGG TGG	243	304-321
Bacterial species:	***Klebsiella pneumoniae***		
2167	5'-CAG CAG CTG GGC GGC GGT	317	36-53

Annex LIV: Internal hybridization probes for specific det ction of *ddl* and *mtl* sequenc s.

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Bacterial species:	***Enterococcus faecium (ddl)***		
2286	5'-AGT TGC TGT ATT AGG AAA TG	2288[a]	784-803
2287	5'-TCG AAG TTG CTG TAT TAG GA	2288[a]	780-799
Bacterial species:	***Enterococcus faecalis mtl)***		
2289	5'-CAC CGA AGA AGA TGA AAA AA	1243[a]	264-283
2290	5'-TGG CAC CGA AGA AGA TGA	1243[a]	261-278
2291	5'-ATT TTG GCA CCG AAG AAG A	1243[a]	257-275

[a] Sequence from databases.